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Contents:

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September 1954 • Jerusalem • Volume IV No. 2

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יוצא לאור ע"י

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BULLETIN OF THE RESEARCH COUNCIL OF ISRAEL

ד. מ. י"ם 2/1677/9.54/1500

In view of the ever increasing amount of material submitted for publication in the Bulletin, it is now felt that some systematization of the contents is necessary. Beginning with Volume V. No. 1, to appear in March 1955, this journal will be published in two sections under separate cover:

A. Exact Sciences

B. Biological and Geo- Sciences

Separate editorial boards have been set up for these sections, and new sections will be opened according to need.

EXECUTIVE EDITOR

NOTICE TO CONTRIBUTORS

Contributors to the *Bulletin of the Research Council of Israel* should conform to the following recommendations of the editors of this journal in preparing manuscripts for the press.

Contributions must be original and should not have been published previously. When a paper has been accepted for publication, the author(s) may not publish it elsewhere unless permission is received from the Editor of this journal.

Papers may be submitted in the following languages: English, French and Russian.

MANUSCRIPT

General

Papers should be written as concisely as possible. MSS should be typewritten, on one side only, and double-spaced with side margins not less than 2.5 cm wide. Pages, including those containing illustrations should be numbered.

The Editor reserves the right to return a MS to the author for retyping or any alterations. Authors should retain copies of their MS.

Spelling

Spelling should be based on the Oxford Dictionary and should be consistent throughout the paper. Particularly geographic and proper names should be checked for approved forms of spelling or transliteration.

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All symbols and text to be italized should be underlined.

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Capital letters should be capitalized in the MS.

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Words to be stopped should be s p a c e d out in the MS.

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Special care should be taken to record clearly relative height of symbols to the line. This is often more easily achieved in legible handwriting rather than typing. Indices and subscripts should be accurately placed. As far as possible formulae should be confined to one line, e.g. $\frac{1}{x}$ should rather be written $1/x$.

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When there is any room for confusion of symbols, they should be carefully differentiated, e.g. the letter "l" and the figure "1"; "O" and "0".

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Decimal division is indicated by use of a full stop on the line, e.g., 1.000 (one, accurate to the third place). Division of thousands is made by use of a comma, e.g., 1,000 (one thousand). Multiplication is indicated by a full stop centrally placed, e.g. $8 \cdot 10^{12}$.

Abbreviations

Titles of journals should be abbreviated according to the *World List of Scientific Periodicals*.

Units are used in the abbreviated form, in the singular, and are not followed by a full stop (only in. is followed by a full stop). The following is a list of the more common symbols: mm cm m km cm³ m³ g mg g kg sec min hr °K °C

Summary

Every paper must be accompanied by a brief but comprehensive summary. Although the length of the summary is left to the discretion of the author, 3 % of the total length of the paper is suggested.

References

Articles

References are to be cited in the text by the author's name and date of publication in paranthesis, e. g., (Taylor 1932). If the author's name is already mentioned then just the year, appears in the paranthesis, e.g., . . . found by Taylor (1932). . . . They are to be arranged in alphabetical order and the following form should be used:

3. TAYLOR, G. I., 1932, *Proc. R. Soc.* London, A138, 41.

Book references should be prepared according to the following order:

4. JACKSON, F., 1930, *Thermodynamics*, 4th ed., Wiley, New York.

The title of a paper will appear in the references only if it has been published solely in a local Hebrew journal. This is printed in regular type and is indicated by double inverted commas.

Letters to the Editor

In Letters, references are to be cited in the text by numbers, e.g., Taylor[3] and are to be arranged in the order in which they appear in the text.

TYPOGRAPHY

In all matters of typography the form adopted in this issue should be followed. Particular attention should be given position (of symbols, headings, etc.) and type specification.

ILLUSTRATIONS

Illustrations should be sent in a state suitable for direct photographic reproduction. Line drawings should be drawn in large scale with India ink on white drawing paper, bristol board, tracing paper, blue linen, or blue-lined graph paper. If the lettering cannot be drawn neatly by the author, he should indicate it in pencil for the guidance of the draftsman. Possible photographic reduction should be carefully considered when lettering and in other details.

Half tone photographs should be on glossy contrast paper.

Illustrations should be mounted on separate sheets of paper on which the caption and figure number is typed. Each drawing and photograph should be identified on the back with the author's name and figure number.

The place the figure is to appear should be indicated in the margin of the MS.

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Authors making revisions in proofs will be required to bear the costs thereof. Proofs should be returned to the Editor within 24 hours. Otherwise no responsibility is assumed for the corrections of the author.

REPRINTS

Each author will receive 50 reprints free of charge, and additional reprints, may be ordered at the time the first proof is returned. A table designating the cost of reprints is sent with the first proof, but may also be obtained upon request.

CORRIGENDA

Vol. III No. 4. p. 443

Measurement of Refractive Index of Absorbing Materials, J. H. JAFFE, *The Weizmann Institute of Science, Rehovot.* Measurements on the refractive index of highly absorbing liquids have been made in this laboratory by interferometric and other methods. It has been found experimentally that an accurate value for the refractive index can be obtained only if the absorption is known. On closer inspection it appears also that the absorption cannot be determined without knowledge of the refractive index (e.g. to correct for reflections at the boundary of the sample. In every case we have examined in practice it turns out that neither the refraction nor the absorption can be obtained singly, but only both together by means of a suitable pair of observations.

Vol. IV No. 2. p. 167- 4 from bottom should read:

Garua (determined by Schulze) which we . . . . .

p. 170, add to References:

5a. DELPY, L., 1949, *Ann. Parasitologie*, **24**, 469.

27a. SCHULZE, P., 1936, *Zool. Anzeiger*, **126**, 258.

In memory of our great friend Max Ball

A STRUCTURAL CONTOUR MAP OF ISRAEL (1:250,000) WITH REMARKS ON ITS DYNAMICAL INTERPRETATION

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INTRODUCTION

Detailed geological mapping has been carried out in the Negev during the last five years (Bentor and Vroman 1951-1954). As the final publication of the geological maps on a 1:100,000 scale will still take considerable time, it seems appropriate to deal in the meantime with some of the more general conclusions of this survey. The tectonic data contained in the appended 1:250,000 structural contour map are only preliminary; more detailed contour maps will accompany the 1:100,000 geological maps.

On trying to explain the tectonic pattern of this area, it immediately became evident that no satisfactory interpretation could be arrived at by limiting the scope arbitrarily to the Negev alone. In fact, the tectonic features of this area are an integral part of a much larger unit and can be understood only within the frame of the more general picture. The contour map was consequently extended to include the northern half of the country. Whereas the map of the Negev is based on the field data of the authors, the map of the northern part of the country is in addition based on maps by G. S. Blake, P. Grader, R. Hanreck, E. Kashai, D. Rabinowitz and Z. Shiftan. The area of Cisjordan beyond the border of Israel and northern Carmel are based on the relatively undetailed maps of G. S. Blake alone; these parts of the contour map are, therefore, the least accurate. As key horizon, the boundary between the Senonian chalk and the underlying Turonian limestone and dolomite was chosen. This boundary is everywhere easily recognized; it is not too much eroded, and generally not too far below the surface; moreover, it is essentially prior to most of the folding periods.

MAIN TECTONIC FEATURES

The main element in the tectonic structure of the area can be described as a huge "S"-shaped fold-range. *The middle stem* of this range, trending N—S, and in places slightly E of N, is formed by the Hebron anticline in the south, the Judean and Ephraim anticlines, continuing into the Buqei'a anticline in the middle, and, after an interruption by the broken zones of the Beisan and Yezreel Valleys, by the Galilean anticline in the north.

Received July 1, 1953.

Every part of this fold zone shows its peculiarities. The Hebron warp is a simple arch with a very broad and flat axial area and relatively steep limbs — somewhat steeper to the west than to the east. The Judean-Ephraim part of the fold range is similar in structure, but bends in its northern half towards the north-northeast (Buqei'a anticline). The Judean fold is still steeper on the western side. The Ephraim fold, however, is steeper on its eastern flank, whereas on its western flank there occurs a series of noses almost at right angles to the main fold, which protrude in an east-westerly direction into the foothill belt. The more conspicuous of these ridges are those of Barfilya, Lydda, Naballa, 'Azzun, Tulkarm. In its Galilean sector the anticlinal warp is much affected by faulting. A very large number of faults, most of them trending E—W, dissect the fold without, however, obliterating the dominant fold feature. The axis of the main warp is readily recognized on the geological map by outcrops of Lower Cretaceous at Beit Netofa, Mughar and Rama. West of this line the strata dip as a rule westwards, and east of it eastwards, even if the individual fault blocks have been markedly tilted during the faulting.

At about the northern border of Israel there starts the *northern bend* of the "S"-shaped fold range. From the northern border of the Hula Depression the fold ranges spread out in a fan-like manner in what may be termed the *Hula Virgation*. The main folds forming the fan are the Lebanon Fold, trending NNE, Hermon and Antilebanon trending NE, and the northern and southern Palmyrean Chains trending NE to ENE.

The *southern bend* of the "S" is formed by the folds of the Northern Negev and the Northern Sinai. Coming from the north, the first indications of the bend are found in the southern periclinal end of the Hebron Arch. This N—S directed arch splits here into two folds: the Dahariya Branch, whose direction is already SW, and the Malhata (Quseife) Branch, directed SW to WSW. The main components of the southern S-bend are the folds of the Northern Negev, such as the ranges of Imara, Yeroham, Hathira and Hatsera, which continue westwards into the Northern Sinai as the Jebel Moghara and Jebel Yelleg—Jebel Hillal Folds. These folds are all built according to a similar pattern: as has been pointed out by many authors, they are all markedly asymmetric, being very much steeper on their south-eastern flank. The asymmetry is the more accentuated as the compressional movement is more pronounced. A second, not less characteristic but generally overlooked, feature of these folds is the convexity of the fold axes towards the SE, this feature again being most pronounced in the strongest folds, viz: those lying further to the SE.

It is important to note that none of these folds cross the 'Araba—Dead Sea Depression. As the fold lines approach, in their north-easterly course, the border of the Areat Rift Valley, they either swing north, becoming parallel to the graben faults, or flatten out and disappear. These Northern Negev folds also form a virgation which may conveniently be called the *Dead Sea Virgation*. In fact, from a compressed area near the SW corner of the Dead Sea these folds fan out towards the SW over an increasingly large area of the Northern Negev towards the 'Avdat Plateau. Between these major fold lines and the edge of the graben proper a particular phenomenon occurs: along the western shore of the Dead Sea and from there southward to the Paran (Jirafi) Valley some twenty brachyanticlines have recently been mapped. Some are elongated in a N—S direction, others are nearly circular. Most of them are of small dimension, covering only a few square kilometres each, but they are pronounced tectonic features

with dips frequently exceeding 30° to 40°. It seems significant that a series of very similar brachyanticlines has been observed in the Sinai, where they are situated between the large folds of the north and the rigid and unfolded plateau of the Central Sinai (Schuermann 1945 fig. 4.) and identical features are known from the Gulf of Suez area.

This, then, is the S-shaped backbone of the tectonic structure. South of it, in the *Central Negev*, a different picture obtains. This area, from the 'Avdat Block and Zin Valley in the north to the Hiyon Depression in the south, is characterized by a series of warps and faults which in many respects are different from the Northern Negev fold ranges. In particular, the following differences can be observed:

(1) In the Northern Negev ranges fault features are of minor importance. The few faults which do occur are of neither great length nor throw and are directed perpendicularly or obliquely to the main fold lines. The Central Negev folds, on the contrary, are without exception bound to long and continuous fault lines, most of them showing throws of many hundreds of metres. These fault lines run sub-parallel to the main folding axes. As examples, the Ramon, Mount 'Arif (Jebel 'Ureif), Mount Sagi (Jebel es-samawa) and Paran fault lines with their associated foldings may be cited.

(2) The Northern Negev ranges are long and continuous fold lines of simple internal structure. Some of the Central Negev folds, such as the big Ramon and the much less conspicuous Badad range, it is true, are still similar, but their structure is already somewhat complicated by a splitting up of the fold axes and the occurrence of numerous domes superimposed on the main fold lines. More typical, however, for the tectonics of the Central Negev are features such as the Mount Sagi and the Paran ranges, which should be described as an irregular row of domes and brachyanticlines along a fault zone rather than as anticlinal folds.

(3) While, as has been pointed out already, the Northern Negev folds approaching the Rift Valley fall into line with the N—S direction of the Rift faults, the faults of the Central Negev and their accompanying folds trend ENE or due E and thus thrust right into the graben. The Paran range, in fact, approaches the graben at a right angle and protrudes straight into it.

South of this dissected area of the Central Negev there is one more fold range: the Tsenifim (Sinaf) Anticline. This may be termed normal; it is a simple fold, affected by minor faulting only, trending N—S and asymmetric in its structure, the eastern limb being by far the steeper one.

In the *southernmost tip of the Negev*, finally, from the head of the Gulf of Eilat to the 'Uvdah (Iqfi) Graben, practically no folding elements can be detected any more, with the exception of the two domal upwarps of Timna' (Menayeh) and Mount 'Amram (Hindis), with their crystalline core. Both are situated on the Rift Graben border and are truncated by the Graben border faults. On the whole, this southernmost area is characterized by a large number of faults, many of them of very considerable throw, surpassing frequently 1000 m. These faults dissect the area into many separate rigid blocks.

DYNAMIC INTERPRETATION

How, then, can the tectonic pattern whose rough outlines have just been sketched be dynamically explained? Since no dynamic interpretation of complex tectonic

structures can ever be subjected to exact experimental proof, the following hypotheses are offered with all the caution demanded by the nature of the subject.

(1) The fundamental assumption that the main folding push has come from the W seems to be well attested by the N—S direction of the largest folds, those comprising the stem of the big "S". The same direction recurs in the isolated Tsenifim Anticline in the far South. This push direction is furthermore reflected in the asymmetry of the Northern Negev folds with their steep SE flanks and still more by the convexity of their fold line towards the SE. But then, these folds stand obliquely to the direction of the main push. The first question, therefore, is: what is the reason for the southern bend of the big "S" forming the Dead Sea Virgation?

A glance at the geological map makes it evident that this bend of the fold range is completely determined by the configuration of the border of the Transjordan—Sinai Massif which acted as a wave-breaker of the Northern Negev folds pressed against it from the W. No fold crosses the 'Araba or the Dead Sea depression, and it is evident that Transjordan reacted tectonically as an independent rigid block, different from the plastic region W of the Rift Valley.

It would be a mistake to regard Cisjordan simply as a continuation westward of the Transjordan block, separated from it by the Rift Valley. In fact, Cisjordan represents a downbroken step between the higher Transjordan Plateau in the E and the still deeper lying area of the Mediterranean in the W. This lower position of Cisjordan in relation to Transjordan is very conspicuous in the geological map, where the crystalline basement is seen outcropping east of the Rift Valley along almost the whole length of the Wadi 'Araba up to the SE corner of the Dead Sea, whereas west of the Big Graben the outcrops of the basement stop practically at the head of the Gulf of Eilat, 150 km further S. The crystalline outcrop on the E-side of the Wadi 'Araba represents the upturned edge of the Transjordan—Syrian basement, which is inclined towards the N and E. South of the Dead Sea the contrast between the plastic area W of the Graben and the rigid block with its relatively thin sedimentary cover E of it is very conspicuous. The inclination of the Transjordan-Syrian basement towards the N, however, with its increasing thickness of sediments, leads to a gradual decrease northwards of the contrast between plasticity in the W and rigidity in the E, until N of the Dead Sea there appear on the Transjordan side the first folds comparable in size and direction with those on the W-side, and north of the Hauran basalt fields the fold ranges of the Anti-lebanon and the Palmyrean Chains spread freely to the E.

(2) The parallelism between the border of the Transjordan-Sinai basement and the direction of the fold ranges, stressed in the foregoing, reflects only a formal relationship. The mechanically effective cause is of course the thickness of the sedimentary column overlying the basement, which in turn is partly determined by its distance from the massif. It is therefore interesting to note that the S-shaped fold range *follows closely the direction of the isopach lines*. In other words, the folds are confined to the area of a certain sedimentary thickness which alone allows the development of long and continuous anticlinal folds. Moreover, the gradient of the sedimentary thickness is very steep from Hebron to the W, but beyond the bend of the isopachs further south this slope decreases, and consequently the folds virgate between the Dead Sea and the 'Avdat Plateau.

(3) A similar explanation may be applicable to the *Hula Virgation* in the N. Here again, the direction of the folds tends to follow the border of the Syrian Massif, which runs from the Gulf of Alexandretta in an East-North-easterly direction. It may, therefore, be suggested that these folds avoid the extremely thick sedimentary column further N, just as the Northern Negev folds avoid the very thin sedimentary cover further S.

(4) Another feature which can now be understood is the *belt of small brachyanticlines* and domes which occur along the western shore of the Dead Sea and hence south to the Paran Valley (Hemar (Muhawwat) Dome, Zohar (Zuweira) Dome, Ashalim Nose, Gidron (Iqfiqfi) Dome, Ein Yahav Dome, Mount Gevim (Ras el-Jeib) Double Dome, etc.). These tectonic forms tend to develop in areas of very moderate sedimentary thickness, near the rigid counterblock, in a position partly sheltered from the direct impact of the tectonic forces by the larger ranges themselves. That such features may develop in areas of very small sedimentary thickness — a few hundred metres — is well attested by the occurrence of the perfectly formed domes of Timna' and 'Amram in the far south, where the basement itself is exposed, partly slightly infolded and partly thrust up into the sediments by faulting.

(5) The northernmost faults in the Negev which show a clear W—E trend occur on the southern plunge of the Northern Negev folds along the Zin Valley, where the highly folded area of the Northern Negev abuts very abruptly upon the 'Avdat Plateau which, at least in its Eocene cover, is practically unfolded. On analyzing the pattern of the contour lines along the northern edge of the 'Avdat Plateau, the strong impression prevails that here the folds of the Northern Negev (Boqer, Hathira, Hatsera) were held stationary for a considerable time, until the tangential strains became so great that the fold ends were torn off and started slipping eastwards (Figure 1). To focus attention

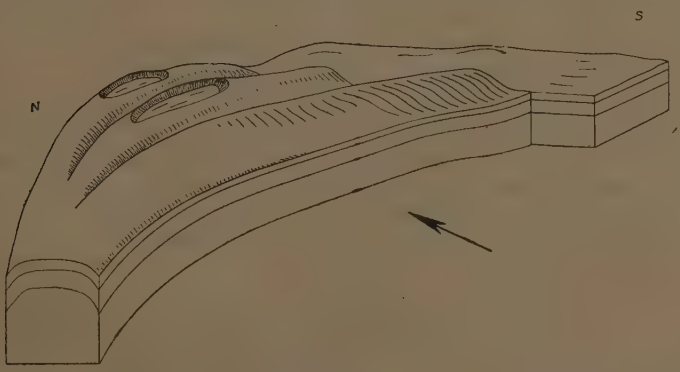

Figure 1
Diagrammatic representation of the Zin Valley strike-slip fault

only on the vertical displacements which doubtlessly also occurred would be to miss entirely the real tectonic significance of these fault lines. Essentially they originated as *strike-slip faults*, i.e. as faults of horizontal displacement. There seems no way of escaping this conclusion when it is considered that the fold ranges north of the Zin

Valley were many kilometres more compressed than the resistive 'Avdat Plateau, south of this line. Along the Zin Valley the folded Northern Negev has thus been torn off from the less plastic Central Negev.

(6) Once this interpretation of the Zin Valley line has been accepted, there seems little reason not to apply the same explanation also to the similar, though much more continuous, parallel fault lines of the *Central Negev*, such as the Ramon, Mount 'Arif, Mount Sagi and Paran faults. Under the fundamental assumption of a uniform stress direction over the whole area, i.e. a compressional stress coming generally from the W, broken folds like those of the Ramon and Mount Badad stand obliquely to that force (in contrast to the Hebron Anticline on which the force gave a frontal push). However, folding obliquely to a force, i.e. *shear folding*, is frequently accompanied by *shear faulting*, in this case strike-slip faulting.

In this respect there is some resemblance to the structure of the Scottish Highlands (Great Glen faults, etc.) which, like the Central Negev, are sliced into parallel blocks by strike-slip faults. But since that part of the world is much more rigid than the Central Negev, the fault-pattern was not accompanied by folding as it was in the Negev. Both patterns would, however, probably become similar, if it were possible to lift the whole sediment mantle from the Central Negev.

(7) These strike-slip faults probably go deep into the basement. There may be even deep lying faults of this type which have not worked their way up to the surface through the softer sedimentary cover. A good case in point seems to be the row of small brachyanticlines of Mount Hamran—Mount Nafha—Mount Sa'ad, which occur on the stable 'Avdat Plateau. These features cannot be regarded as forming an ordinary fold line, such as for instance the Hatsera Anticline. It seems much more probable that they show the reaction of the sediment mantle to a horizontal movement of the basement along a buried strike-slip fault. This fault in fact appears on the surface somewhat further east — in the area of Mount Teref. Toward the W, the fault passes into a very narrow V-shaped syncline, which occasionally is broken into a graben. This scar runs immediately south of the aforementioned brachyanticlines.

(8) This explanation becomes even more probable if we compare the Hamran—Sa'ad line with those places in the Central Negev where the strike-slip faults are well exposed on the surface, such as the Mount Sagi and Paran faults. These faults are beset on both sides with a series of small brachyanticlines, the axes of which do not run parallel with the fault but stand somewhat obliquely to it; they are shear-folds oriented parallel with the long axis of the strain ellipsoid, developed along shear-faults (Fig. 2). In

Figure 2
Shear-folding along strike-slip faults

this connection it is interesting to note that sometimes the folds are only half-domes developed on one side of the fault only. Such cases have been observed in the so-called "Triassic Dome" of the Ramon, and on some domes along the Mount Sagi line.

(9) The Northern Negev was plastic enough to allow the folds approaching the Transjordan Massif to bend north. The folds of the less plastic Central Negev behave differently. Here, the folds, moving east with the dissected blocks at almost right angles to the Transjordan Massif, were *squeezed flat against it*. This is well seen in the Badad Anticline, whose eastern nose has been pressed against the Transjordan wall and squeezed into a flat front.

The case of the Ramon Anticline is even more illustrative (Figure 3). This anticline came up in its eastward move against the belt of brachyanticlines, represented here by the 'Ein Yahav, 'Ein Rahel and 'Irayis—Shahak domes. As pressure from the W continued, it found relief only north and south of these obstacles, and the fold was split into two components which moved NE and SE along two strike-slip faults: the Masor

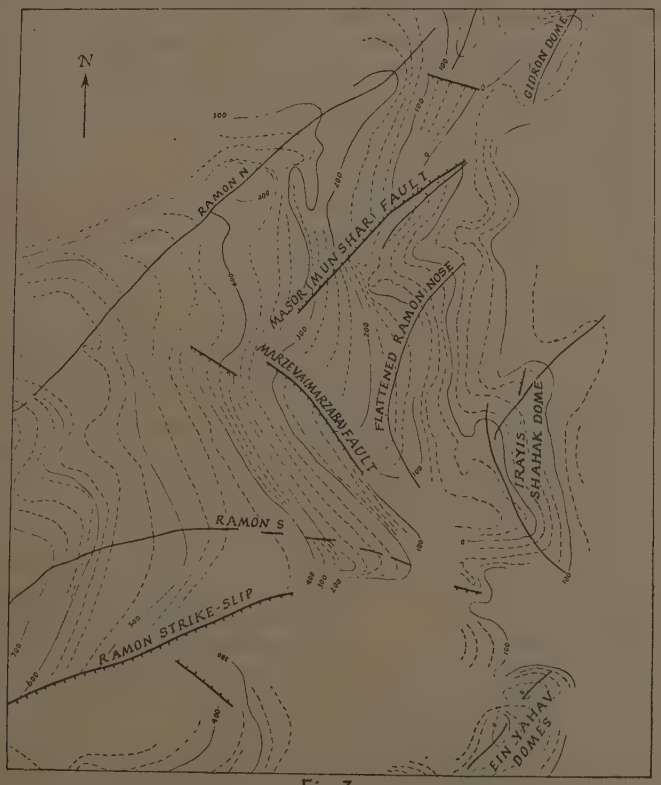

Fig. 3

Contour map showing details of structures in the eastern part of the Raman Anticline.
Scale 1: 200,000

(Munshar) fault, trending NE, and the Marzeva (Marzaba) fault trending SE. Both faults link up westwards with the Sa'ad—Hamran strike-slip.

(10) The tectonics of the Central Negev from the Mount Sagi and Paran lines in the south to the Zin Valley in the north are therefore determined by the still moderate thickness of sediments, which moreover were made more resistant through widespread magmatic intrusive and extrusive activity during late Jurassic and early Lower Cretaceous time (Makhtesh Ramon, Makhtesh 'Arif). The result was the slicing of the area into separate blocks through shear-faults, along which rows of brachyanticlines were developed (Figure 2). The separate blocks were moved differentially eastwards.

(11) South of the Paran Valley there occurs one more fold, the Tsenifim (Sinaf) Anticline. This may be called a "normal" fold. Its direction is N—S, its structure asymmetric, with the steep flank on the eastern side; faulting is unimportant. The occurrence of such a "normal" anticline so far south is explained by the large sedimentary basin which developed in this area, at least in Upper Cretaceous time. All the formations to be observed in the Tsenifim Anticline, viz. Turonian, Santonian and Campanian, are each about twice as thick as e.g. in the Northern Negev. This area appears on the isopach map as an island of relatively high sedimentary thickness. In consequence, rocks were folded perpendicular to the direction of compression and pushed against the apparently more stable Hiyon Depression in the E. The northern end of the Tsenifim Anticline bends around the northern end of this stable area. Some less pronounced folds, also directed N—S, such as the 'Uvda Anticline, occur south of the Hiyon Depression.

(12) The distinctive feature of the *southernmost tip of the country* extending from the Gulf of Eilat to and comprising the 'Uvda Depression, is its extreme brittleness. It is in fact a downbroken continuation of the Transjordan Massif, thinly disguised in places by a small sedimentary cover. This sediment mantle has been squeezed into sharp synclines between gliding and possibly also rotating crystalline blocks (Figure 4).

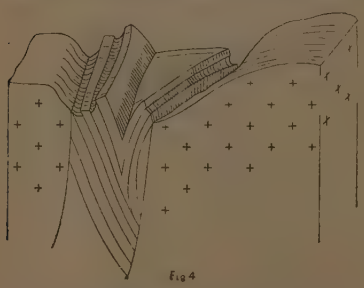

Fig 4

Block-diagram showing infolded sediments between blocks of crystalline basement. Eilat area

It is interesting to note that along the 'Araba border indications of *tensional* stress occur, as exemplified by the Yotam (Isdeirat) Graben, a small sedimentary block downfaulted into the basement complex by much more than a thousand metres. Notwithstanding this large throw, the graben is, in its northern end, only about 10 m wide. Such features cannot be explained by conditions other than tangential tension. In contrast, a certain distance W of the 'Araba Depression conditions of *compressional* stress prevail. This is indicated by the numerous narrow V-shaped synclines into which even such hard rocks as the Campanian flint beds have been squeezed, and by the occasional upthrust of a crystalline block over the adjoining sediments such as, for instance, along the eastern 'Uziahu ('Alaliq) Fault or in the western part of the Timna' Makhtesh.

(13) The case of the Hebron—Judea—Ephraim Anticline, trending N—S, i.e. perpendicular to the main push, is much simpler. Here, a sufficiently thick sedimentary

sequence extended over a wide area. In this anticline all the forces were spent in the folding movement. Such an intense squeeze causes a tendency of elongation of the long axis of the strain ellipsoid, i.e. along the fold axis. An anticline, such as the Central Palestine Fold Range, built in part by relatively hard rocks, such as the Cenomanian limestones and dolomites, shows resistance to this tendency of elongation. As there exists only one direction of relief, i.e. upward, the elongating anticline will start to crinkle into small secondary folds perpendicular to the main axis. This may well be the explanation of the Tulkarm, 'Azzun, Naballa, Lydda and Barfilya noses. Such features are especially pronounced on the western flank of the main arch, but occur on its eastern flank as well (Kafr Malik, El-Buqei'a Nose, also Wadi Far'a Graben [?]).

Sedimentary thickness is much greater here than in the Northern Negev. This difference is well reflected in the structure of the folds. Asymmetry is less pronounced and the fold shows a very large and almost horizontal axial area, clearly distinct from both flanks. In contrast, the Northern Negev folds consist essentially of two flanks only, a very gentle north-western and a very steep south-eastern one.

(14) A last and very important type of movement has yet to be mentioned. In relatively recent times large-scale *broad warping* of the complete fold bundles took place. This is definitely attested, e.g. by the occurrence of marine Miocene at +300 m near Yalu (Avnimelech 1936), at + 400 m in the Yeroham Syncline, and even at + 500 m south of Hebron (Picard 1943). These movements took place partly without rupture, but partly, as for instance over most of the Negev, the strata were lifted along the older strike-slip faults which now assumed a dip-slip character. But it is in the north of the country (and in the adjoining Lebanon) that these warpings gave rise to a particular feature.

The *Galilean Anticline*, as mentioned, is dissected by very many faults of a general E—W trend. Similar features occur, it is true, also in the Judean Arch, e.g. west of Jerusalem. In addition, it seems quite probable that, if the Hebron and Ephraim sectors of the Central Mountain Range were known in as great detail as the Judean part, similar fault lines would be seen to occur there too. The difference between the Galilean Anticline and those further south is, therefore, more one of degree than of kind. The difference in degree, however, is so pronounced that a special cause should be looked for. Moreover, whereas in the Judean Anticline the faults are confined to the central axial area, in the Galilean Anticline they occur as well, and perhaps in an even more pronounced manner, along both flanks of the arch. The cause of this difference seems to lie in the narrowness of the Galilean area. In fact, the distance between the Hula Depression in the E and the fault coast in the W is only 40 km as against 65 km from Tel Aviv to the border fault of the Jordan Valley and 90 km from Gaza to the Dead Sea Graben. During the upwarp movement, the Galilean Anticline was, therefore, strongly held back on both sides as in a pincer with consequent disruption and slicing into separate blocks. This E—W faulting continues, as mentioned, into the Lebanon to the north, where the tectonic position is analogous. Faulting in Galilee is therefore essentially subordinate to and younger than the primary folding. But even here, some of the faults may have originated as strike-slip faults during the folding, as evidenced by the occasional occurrence of horizontal slicken-slides.

In the extreme NE corner of Galilee, W of the Hula Depression, a second anticline occurs, separated from the Central Galilean Arch by the Eocene-filled Marum er-Ras

Syncline. This fold, the *Naphtali Anticline*, just as the main one, has its outcrops of Lower Cretaceous (Manara slope). This fold is truncated by the western border faults of the Hula Depression. It remains, therefore, undecided whether the Naphtali Anticline represents a separate fold, or whether it should be regarded as the westernmost cuesta of the big Hermon Warp, NE of the Hula Depression.

(15) In summing up, *seven tectonic provinces* (marked I—VII on the map) may be distinguished in Palestine. The tectonic pattern changes from area to area, dependent as it is on strength and direction of the compressional movement, distance from the rigid crystalline basement, thickness of the sedimentary cover, and the plasticity of its composing rock sequence. From south to north these areas are:

I. The "*Far South*", a shattered and broken part of the crystalline basement, in part overlain by a thin sedimentary veneer.

II. The *Tsenifim* (*Sinaf*) *Anticline*, a normal anticlinical fold in the centre of the "Sedimentary Basin of the Southern Negev". East of it extends the stable Hiyon Depression.

III. The *Central Negev Uplands*, characterized by strike-slip faults and buckling of the still relatively thin sedimentary cover into rows of domes and brachyanticlines.

IV. The *Northern Negev Fold Bundle* forming the Dead Sea virgation; medium thickness of sediments; arcuate fold lines; highly asymmetric fold forms.

V. The *Hebron Range*, a broadly arched, N—S directed fold, less asymmetric in structure and flat in its central, axial, area.

VI. The *Judean—Ephraim Arch*, similar in structure to the Hebron Range. To the northwest of the main fold range there seem to occur two additional fold lines, trending NE, those of Umm el Fahm and Mount Carmel. These should link up north of the Zevulun—Yezreel Depression with the Galilean Anticline.

VII. The *Galilean Anticline*, strongly block-faulted along EW dip-slip faults.

CHRONOLOGY OF TECTONIC EVENTS

It remains to fit these tectonic events into a historical timetable. The intrasenonian unconformities in the culmination of the Northern Negev anticlines have provided evidence (Bentor-Vroman 1951) for the beginning of the folding movements at least as early as the end of the Turonian. It can even be stated that, at least half of these folding movements were completed in the Middle Eocene. If such unconformities have so far not been observed in the north of the country, the reason is obvious. The same degree of folding which in the south made all the difference between sedimentation and emersion led in the deeper lying north of the country only to a change in depth of sedimentation, a change much more difficult to observe.

Middle Eocene strata were still subjected to folding, but these movements probably stopped before the Middle Tertiary. Predominantly terrestial sediments, such as the Hatseva (Husb) Series, whose base conglomerate is at the latest Middle Miocene, fill basins along the fault lines covering the faults unbroken (Paran—Karkom—(Ubara) Area) or abut horizontally against steeply inclined anticlinal slopes (Oron (Er-Rakb) Syncline). Only very faint traces of post-Miocene folding (as distinct from broad warping) have so far been found anywhere in the country.

Strike-slip movements started at about the same time as folding. In fact, the intra-senonian unconformities in the culmination of the Nafha—Sa'ad shear-folds are completely the same as those elsewhere in the Northern Negev. Continuing throughout the Eocene, they too seem to have stopped before the Middle Tertiary. At that time the compressional strain subsided and, as a counter-action, large-scale broad warping of the complete fold bundles set in. These movements, with the accompanying dip-slip faulting, continued during Upper Tertiary time. It seems that in particular areas such as Mount Carmel these movements are still continuing (personal communication by Y. Itzhaki).

The age of the big Jordan-'Araba Depression is not easily fitted into this scheme. This on the whole N—S directed graben does not follow consistently any major tectonic line. From about the latitude of Jerusalem down to a point about 40 km north of Eilat, the graben coincides more or less with the border of the Transjordan Massif. Further to the north, the graben follows roughly a syncline between two big folds, while in the far south the rift valley follows nothing at all but simply cuts into the basement. It has been shown elsewhere (Bentor and Vroman 1954) that the tectonic instability of this area can be recognized as early as the Cenomanian. In some areas along the graben, as in the Dead Sea region, it can be proved that already in Middle Tertiary times there existed a deep depression, even if it was not necessarily broken at that time. Major faulting occurred probably since Miocene, but the faults were still active in quite recent times, such as the Early Pleistocene.

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THE UM BAREK OIL SHALE

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The study of oil shale as a source of synthetic liquid fuel has raised much interest in recent years in many countries. This mineral is known to occur in Israel, and since the country has at present no domestic fuel production, the investigation of local deposits of this material is of particular importance.

The oil shale deposits of Israel, more commonly designated by the misleading term of "bituminous limestone", have repeatedly attracted the attention of geologists and technicians (Blake 1930, Picard 1931). Several outcrops of the material have been known for a considerable time**. G. S. Blake and W. J. Goldschmidt (1947) and L. Picard (1931) have recorded the occurrence of "bituminous limestone" as found by visual inspection in a large number of water well drillings. The points reported by these authors are marked on the map given in Figure 1. Samples from the well drillings, as far as they were available, were analysed by Gottesman and Yashunsky (1950) and by us. Table I gives our results and includes also data on samples of recent drillings. It can be seen that oil shale is found in many parts of the country. The layers are as a rule of Senonian age and may occur as outcrops in some areas (Safed, Meggido), or at varying depth from the surface in others (e.g. at 100 m in the Beersheva region and at 300—350 m at Har Tuv).

TABLE I

Analysis of oil shale from various locations in Israel

No.	*Location*	*Coordinates* *x*	*y*	*Depth* *m*	*Oil cont.* % (a)	*Org. matter* % (b)	*Remarks*
1	Hulda	138.190	137.750	75 — 80	4.0	8.9	Ref. 2 p. 231
	"	"	"	81.5— 82.7	—	10.2	" "
2	Ayalon No. 3	145.350	142.120	87 —131	7.5	—	From water drillings of Mekoroth Ltd.
	"	"	"	273	1.7	6.2	"
3	Bakaa el Gharbia	202.100	153.600	92	6.0	11.6	"
4	Karkur	207.103	150.570	68 — 85	—	6.5	Ref. 2 p. 181
5	Pardess Hanna	207.410	149.790	136	—	3.8	" p. 184/6
6	Ein Hashopheth	222.540	160.585	42.5— 42.75	—	3.8	" p. 172
7	Meggido			outcrop	3.0	5.7	taken by the authors
8	Ein Zeitim			outcrop	2.3	5.7	"
9	Safed			outcrop	2.4	5.1	"

(a) Determined by a semi-micromethod (about 5 g) according to Salomonsson (1950).
(b) By calcination after treatment with HCl.

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** The important deposits of Nebi Musa, near Jericho, are now outside the area of Israel. Important studies of this area and its oil shale have been made by several authors, notably by G. S. Blake (1930) and L. Picard (1931).

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Some systematic efforts to study these deposits in order to assess their potential contribution to the fuel supply of the country were made in the last five years. The first steps in this direction were the drillings in the Um Barek area carried out in 1950—1951 by the Israel Army Scientific Department in collaboration with the Weizmann Institute of Science*.

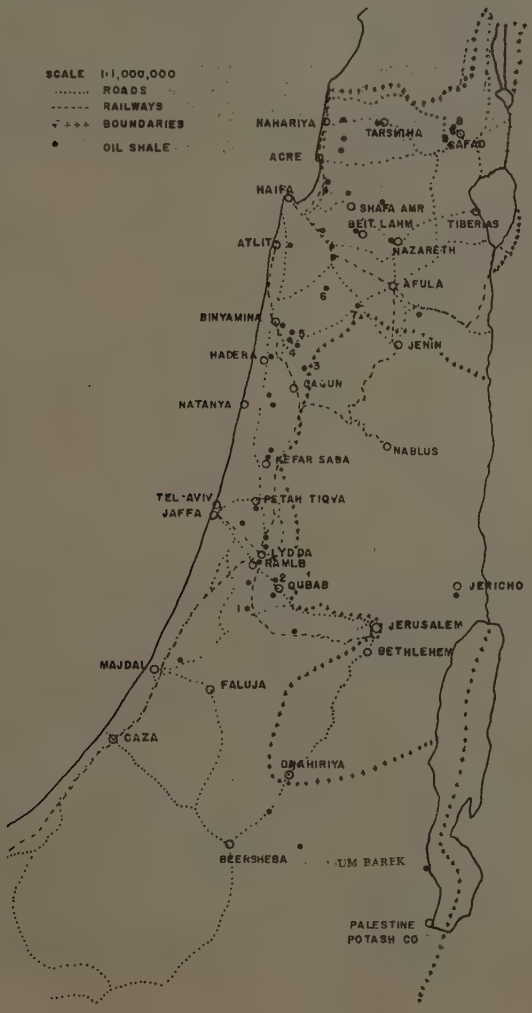

Figure 1. Map of Israel showing oil shale occurrences. The locations marked by numbers correspond to the samples of Table I.

* At a later stage the Research Council of Israel and the Israel Mining Company also participated in the project.

The Um Barek Wadi drains into the south western part of the Dead Sea at a point 15 km north of Sodom. The oil shale layers are according to Y. Vroman (1951) of Senonian age, as in many other parts of the country, and are situated between Maestrichtian flint and Turonian dolomite. Their occurrence has been ascertained over an area extending 3—4 km from south to north and 0.5 km from east to west.

Owing to the complicated topography of the area they may appear as outcrops in some places or be under varying amounts of overburden in others. Also the thickness of the layers varies from point to point, reaching a maximum of about 40 m at G1 and G2 (Figure 2). Accordingly it is very difficult to make an estimate of the reserves of the area, particularly with the meagre data available at present. Provisionally, the amount of oil shale has been evaluated at 60,000,000 tons (Vroman 1951) of 5% average oil content (about 10—12% organic material).

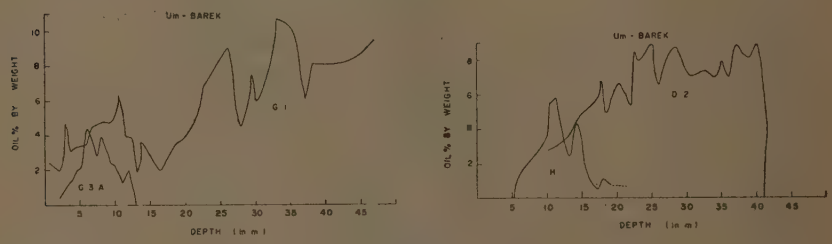

Figure 2
Variation of oil content with depth
a) Bore Hole G1 and G3A b) Bore Hole D2 and H

The samples of oil shale examined were obtained from a number of core drillings, the locations of which are marked on the attached air-photograph (Figure 3). The oil content of the shale, as measured by Fischer Assay (Stanfield and Frost 1949), varied from 2—10% by weight, according to the depth of the layer. (The total organic material is approximately two to two and a half times the amount of Fischer oil). Typical graphs showing the variation of oil with depth are given in Figure 2.

The oil shale of Um Barek is a mineral of grey colour and conchoidal fracture. Its structure is compact and no lamination is apparent on examination with the naked eye.

The main mineral constituent of the oil shale is calcite. However, in some parts of the deposit dolomite replaces the calcite to a varying extent.

The average mineral composition is as follows:

$CaCO_3$ and/or dolomite	50—60%
SiO_2 and silicates	15—20%
P_2O_5	1—4%
R_2O_3 (mainly Al_2O_3)	2—7%
Pyrite	1%

Samples taken from Bore Hole C1 were examined for their mineral constituents by X-ray analysis. The results are given in Table II.

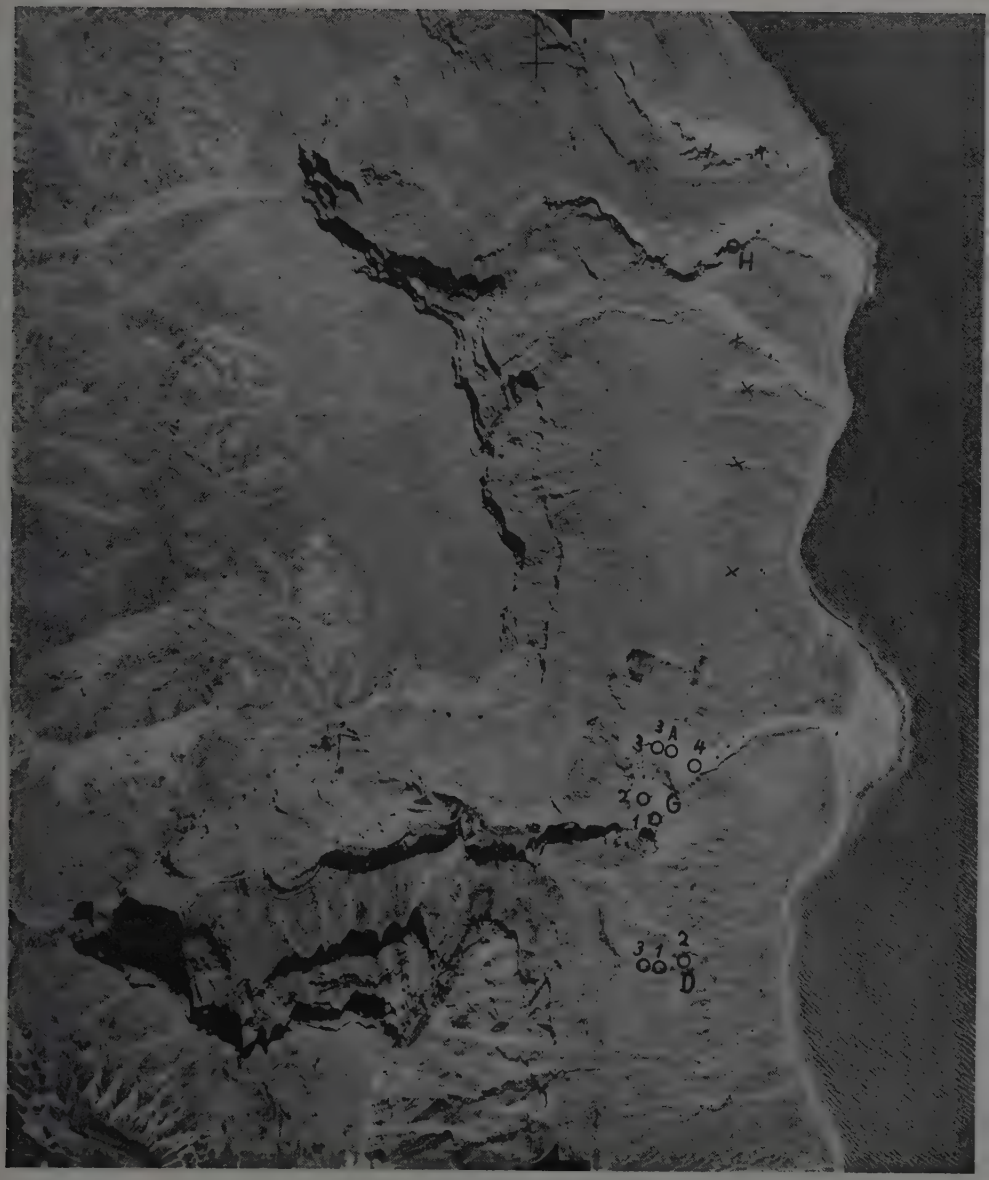

Figure 3
Air photograph of the Um Barek Area Scale : 1:15,000 ○ Bore-hole · Outcrop

TABLE II

Mineral constituents of the Um Barek oil shale

Depth (m)	*Mineral constituents identified*
15—18	Calcite (principally), pyrite, kaolin, quartz, traces of dolomite.
21—24	Calcite (principally), quartz, pyrite, kaolin, hydroxyapatite, dolomite.
47	Dolomite (principally), quartz, kaolin, hydroxyapatite.

The nature of the mineral constituents was further ascertained by petrographic analysis of thin sections. In agreement with the X-ray results it was found that the sample from Gl at 47 m depth consisted principally of dolomite and organic material. The dolomite occurred as irregular grains ranging in size from minute particles up to grains of 0.1 mm. The minor constituents were pyrite, calcite, quartz, clay, probably collophane and feldspar. Granular pyrite averaging 0.01 mm across was scattered throughout the specimen, but no cubic forms of pyrite were observed. Single grains of calcite approximately 0.1 mm across and veinlets of calcite 0.01 to 2 mm across were present. The calcite grains in the larger veinlets were irregular in shape. The veinlets appeared to be a secondary filling of minute fractures. Calcite was also found replacing shell fragments of microfossils. The quartz and feldspar in the sample were irregular subangular fragments. The feldspar was present in by far the smaller quantity, as only a few grains were observed. Clay was disseminated throughout the slides and could not be identified by petrographic means. One oval-shaped, yellowish-brown isotropic material was thought to be collophane.

Spectrographic analysis* showed the presence of the following trace elements: Cr, Sr, Co, Zr, Cu, Vd, Mo, Ni, Ti, Ba, Ga, Na, Li and K.

Uranium** was found to be present to the extent of 0.002—0.003%.

An attempt was made to determine the origin of the organic material by examination of thin sections under the microscope (Figure 4). However, only structureless material.

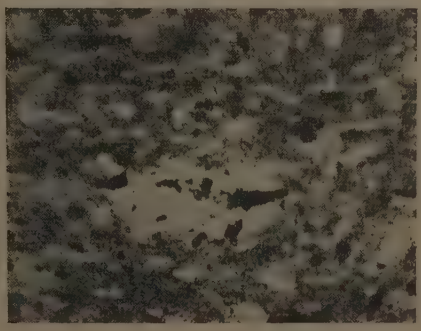

Figure 4

Thin section of Um Barek Oil Shale showing finely divided organic material in a mineral matrix. The darkest spots are Pyrite. Enlargement × 120.

* The analysis was carried out by the U.S. Bureau of Mines Station, College Park, Maryland.
** Determined by the U.S. Geological Survey, Denver, Colorado.

light yellowish brown to brownish black in colour, was found. No spores or algal colonies, as detectable in certain shales, could be seen. The aspect of the thin sections is typical of the deposition of organic debris in marine environment (Brotzen). Remains of many microfossils, particularly Foraminifera, appear on the slides. They are not, however, directly connected with the genesis of the organic material.

The organic material (usually termed kerogen) is soluble in organic solvents to a small extent only and thus behaves in a way typical of true oil shales. Results of a number of extraction experiments of a sample from GI at 47 m depth are given in Table III. The extraction gave a solid varnish-like material and not a heavy liquid as in the case of other oil shales.

TABLE III

Extraction of the Um Barek Oil Shale

Solvent	*% by weight extracted*	*% by weight of the org. matter*
n-Hexane	.6	3
Carbon tetrachloride	.8	4
Thiophene	1.2	6
Pyridine	2.2	11
Morpholine	4.5	22

The organic material can be isolated by leaching with HCl and HF, followed by reduction of the pyrite, according to Himus (1942). However, the ash content cannot be reduced below 3—4% by this method. The kerogen of the Um Barek Oil Shale seems thus to be very intimately mixed with or even bound to part of the inorganic constituents, as in other cases (e.g. the Scottish Oil Shale) the ash content of the organic material may be reduced to 0.7—0.8% by the method of Himus.

The organic material, isolated by leaching, is a brown blackish powder, which does not exhibit any definite crystal structure by X-ray examination. A sample of kerogen (from Bore Hole D at 22—23 m) which still contained 3.4% of ash was submitted to elementary analysis and the following composition was found:

C 65.2, H 6.6, N 3.0, S 13.2, O 12.0 (by difference).

One has, however, to keep in mind that treatment by strong acids may degrade the kerogen. The ultimate composition of the organic material can also be estimated from the total organic content and the amount and elementary analysis of the assay products. Thus for a sample from GI (at 47 m) the following figures were obtained:

C 69.1, H 8.6, N 1.9, S 11.0, O 9.4 (by difference).

The deviations of the two sets of figures may be due to local variations of the shale, but also to systematic errors involved in both methods.

The most notable feature of the kerogen is its high sulphur content, which seems to be characteristic not only of the Um Barek material, but of Israeli shale in general. The nitrogen content, too, is considerable. On heating the shale to 400—500°C, the kerogen decomposes into oil, water, gas and coke (which remains in the ash).

The assay products form approximately in the following yields:

Oil	30—50% (b.w. of kerogen)
Water	10—15% "
Gas	10—15% "
Coke	35—40% "

The elementary analysis of a number of assay oil samples gave the following average figures:

C 78—82, H 9.5—10.5, N 0.8—2.0, S 6.0—7.0.

The specific gravity varied from 0.950—0.975 g/ml. The heat of combustion of one sample was found to be equal to 9450 cal/g. As could be expected from the composition of the kerogen, the nitrogen and sulphur contents were high.

It was shown qualitatively that the oil contained phenolic and pyridine-like substances. Wax was present in small amounts only (Shabtai). Some gas compositions determined by mass spectrometer analysis are given in Table IV. As in the case of the oil, the assay gas is characterised by a very high sulphur content (H_2S up to 38 %).

TABLE IV

Gas from the assay of oil shales from Um Barek, Bore Hole G1.

Depth (m)	[1] 15—18	[1] 21—24	[2] 47
Yield of gas (dry, air and helium free, at 60 °F and 760 mm Hg pressure)			
Litres per 100 g of shale	1.527	1.916	[3] 1.374
Specific gravity (calculated from composition), air=1.000.	1.153	1.124	1.079
Composition, volume percent			
Methane	12.6	14.5	13.3
Ethane	4.7	5.9	6.3
Propane	1.5	1.5	1.8
Butanes (*n*- and iso-)	1.2	1.4	2.8
Pentanes (*n*- and iso-)	.7	.9	.7
Ethylene	1.5	1.4	1.6
Propylene	1.8	1.5	1.2
Butenes (1, 2 and iso)	1.4	1.3	1.6
Pentenes and cyclopentane	.8	.6	1.5
Hexenes and/or cyclohexane	.4	.4	.4
Butadiene	.1	.1	.1
Pentadienes and/or cyclopentene	.0	.2	.1
Benzene	.0	.0	.3
Carbon dioxide	29.0	27.3	13.6
Carbon monoxide	6.0	4.3	5.6
Nitrogen	.0	2.2	—
Hydrogen	7.8	8.4	11.0
Hydrogen sulphide	30.5	28.1	38.1
Total	100.0	100.0	100.0

1) Collected over acidified solution of sodium sulphate.
2) Collected in evacuated flask and corrected to nitrogen-free basis due to contamination with air.
3) Volume determined by duplicate assay and collection of gas over acidified solution of sodium sulphate.

The source of the sulphur compounds in the gas and the oil seems to be both the organic sulphur of the kerogen and the pyrite present among the mineral constituents. The distribution of sulphur in the oil shale and its assay products, as found in three different samples, is shown in Table V.

TABLE V

Distribution of sulphur in oil shale samples from Um Barek and in their assay products, Bore Hole G1,

Depth (m)	15—18	21—24	47
Sulphur content, percent of raw shale			
Assay oil	0.2	0.4	0.7
Spent shale	1.5	.9	.7
Gas (by difference)	1.1	1.0	1.3
Total	2.8	2.3	2.7
Types of sulphur, percent of total sulphur in raw shale			
Sulphate sulphur	14.9	13.6	8.5
Pyrite sulphur	41.3	21.3	14.0
Organic sulphur (by difference)	43.8	65.1	77.5
Total	100.0	100.0	100.0
Assay oil			
Organic sulphur	7.8	17.0	25.1
Assay gas (by difference)			
Organic and/or mineral sulphur	37.7	43.0	47.6
Spent shale			
Mineral sulphur (pyrite, sulphide & sulphate[1])	26.0	17.5	11.4
Organic sulphur	28.5	22.5	15.9
Total	100.0	100.0	100.0

1) Contained not more than 0.1 percent sulphide sulphur; little or no H_2S was evolved by treatment with dilute acid.

Both the high proportion of organic sulphur and the percentage of sulphate are notable. Contrary to the observations made on Colorado Oil Shale (U.S. Bureau of Mines), the percentage of pyrite sulphur diminishes on retorting, which means that it contributes to the formation of the sulphur compounds found in the assay products.

The heat of combustion of a number of oil shale samples is given in Table VI. These data are of particular importance in view of the fact that the direct combustion of shale, e.g. under boilers for the production of steam and electricity, seems of interest in Israel. The accurate determination of the heat of combustion presents a number of difficulties. In particular it is necessary to leach the material with acid prior to combustion in order to eliminate errors due to partial decomposition of calcium carbonate. Corrections have also to be made for sulphate formation. The figures are estimated to be accurate to ± 10 cal. The heat of combustion is expressed in cal/g of the original material and the calcium carbonate is assumed to remain undissociated. The values found vary from 630 to 1550 cal/g, according to the richness of the material.

TABLE VI

Heat of combustion of oil shale from Um Barek

Sample from Um Barek	*Heat of combustion cal / g*	*% oil b. w. (Fischer Assay)*
G1 12—15 m	630	3.6
G1 12.7 "	640	3.9
G1 28.9 "	1550	7.5
G1 47 "	1480	9.6
Outcrop near G1	1270	7.5

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A BACTERIA-FREE CULTURE OF *PRYMNESIUM PARVUM* (*Chrysomonadina*)

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In 1946 the appearance of a *Chrysomonadina*, *Prymnesium parvum*, in our fish ponds in brackish water led to mass mortality of the fish and caused serious damage (Reich and Aschner 1947). It was clear that an exact analysis of the biological requirements of this flagellate would constitute the best basis for its control.

The senior author therefore undertook an attempt to obtain a bacteria-free culture of the flagellate.

The investigation was carried out in four stages: 1. Establishment of enrichment cultures; 2. Elimination of other algae and protozoa from the culture; 3. Elimination of accompanying bacteria; 4. Determination of the conditions necessary for growth.

I. Establishment of enrichment cultures

Unsuccessful attempts at culturing were made with 1) pondwater, sterilized in an autoclave; 2) 10—15 per cent natural or artificial sea-water, to which had been added varying amounts of Killian's nutrient solution as well as individual nutrient salts. The number of flagellates decreased with each successive transfer, and after three to four transfers they were usually outnumbered by other organisms which had been present in the original pond water. From these experiments we gained the impression that mass development of *Prymnesium* in our fish ponds was dependent on the presence of a certain substance whose increasing dilution in the culture media led to progressive decrease in the number of *Prymnesia* and, eventually, to their complete disappearance. All attempts to replace the missing growth substance by the addition of organic substance (peptone, amino acids, sugar, or fatty acids) resulted in an increased growth of the accompanying bacteria and thereby accelerated the disappearance of the flagellates.

We then attempted to employ diluted sea-water from various sources as medium. Sea-water from aquaria in which *Artemia salina* had been raised for an extended period was found to stimulate the multiplication of *Prymnesium*. Even better results were obtained when small amounts of dry yeast (one part yeast per 20,000 parts solution) or peptone (one part peptone in 100,000 parts water) were added to those cultures. *P. parvum* increased very quickly in such cultures and in ten or twelve days reached a population density of more than one million cells per one ml of solution, this being a concentration found in our ponds only in exceptional cases.

In this way cultures were established in 250 ml Erlenmeyer flasks. They remained in good condition for months at about 25°C room temperature in diffuse daylight and served as a starting point for the successive experiments on purification.

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II. ELIMINATION OF OTHER ALGAE AND PROTOZOA FROM THE CULTURES

Our earliest experiments already showed that *Prymnesium* in young and vigorously growing cultures showed a negative geotaxis and a positive phototaxis. Thus the flagellates initially collected in a semi-circular, superficial layer on the illuminated side of the culture vessels, later spreading in a thin layer over the whole surface. *Prymnesium* distributed itself more evenly through older cultures, and in very old cultures the flagellates aggregated in a thick layer on the floor of the vessel.

The taxis of the young *Prymnesium* cultures was used as a means to separate the flagellates from other algae and protozoa. Ten ml of the nutrient solution were sterilized in a test-tube and then inoculated with 0.5 ml of the enrichment culture. In order to accelerate the accumulation of *Prymnesium* on the surface, the lower part of the test tube was wrapped in black paper and the culture was placed on a window. After two days a brown ring of the accumulated flagellates appeared on the side facing the light. With the aid of a capillary pipette small numbers of flagellates were transferred to fresh medium from these concentration rings. Several repetitions of this procedure separated *Prymnesium* from all other algae and protozoa.

III. ELIMINATION OF BACTERIA FROM THE CULTURES

New mass-cultures were established in Erlenmeyer flasks from the one-species cultures obtained by the methods described in section II. The nutrient medium was modified as follows: since cultures in the original medium showed an acid reaction after a few days, which led to decreased multiplication of *Prymnesium*, 5 parts Na_2HPO_4 and one part $NaNO_3$ were added to 100,000 parts of solution which was then brought to pH 9.2 with NaOH. Furthermore, in order to provide uniform conditions for development, natural light was replaced by artificial illumination with a 32 watt fluorescent lamp.

Cultures were first treated with various concentrations of penicillin. This did not eliminate bacteria, since more than 500 units/ml penicillin killed *Prymnesium*, while many bacteria remained alive at lower concentrations.

We therefore treated cultures successively with various antibiotics. They were first passaged through three cultures containing 0.1 mg/ml sulfanilamide, then through sulfathiazole (0.1 mg/ml), streptomycin (1 mg/ml), and finally again through penicillin. This procedure showed that, contrary to the response of untreated initial cultures, *Prymnesium* cultures which had already been freed of most bacteria were able to withstand up to 2000 units/ml penicillin. They required transfer to a fresh penicillin solution after no more than 48 hours of developmemt, since the flagellates died if left for a longer period in the unchanged penicillin solution.

This response indicates that the toxic effect on *Prymnesium* was due not to the penicillin itself but rather to by-products formed in the solution. After two or three passages through the penicillin solution, bacteria-free cultures of *Prymnesium* were obtained, which multiplied vigorously in the penicillin-free culture medium.

IV. CONDITIONS NECESSARY FOR GROWTH

a) *Standard cultures*

The bacteria-free cultures were grown in a medium similar to the one described in section III. Natural sea-water was, however, replaced by an artificial preparation according to Brujewicz (cited in Sverdrup, Johnson and Fleming 1942). In order to bring the

total salt content of this solution to a concentration more favourable to *Artemia salina*, 5 g NaCl per litre were added to this sea-water. *Artemia* fed on dry yeast were maintained in this solution for long periods before the medium was used in *Prymnesium* cultures. For the *Prymnesium* culture, 30 ml of such a solution were diluted with 70 ml of distilled water, and to this were added the above mentioned amounts of Na_2HPO_4 and $NaNO_3$ and one part/mil. Difco-proteose-peptone. The solution was brought to pH 9.0, and sterilized in the autoclave.

Cultures were generally grown at 20°C in a thermostat, in tilted test-tubes containing 5 ml of the medium, which were illuminated 14 hours a day with a 32 watt fluorescent lamp. Every three weeks 0.5 ml of a culture was transferred to fresh nutrient medium. Cultures maintained in this manner reach their population maximum after 12 to 14 days. They have been serially transferred for 18 months without showing any decrease in reproduction or any other damage to the flagellates.

b) *The organic substances necessary for growth of* Prymnesium

The behaviour of *Prymnesium* in the enrichment cultures showed that, in spite of the well developed chromatophores, we were dealing with a distinctly heterotrophic organism. Experiments were therefore carried out with bacteria-free cultures to determine more closely its organic requirements.

The first six series of experiments contained the following combinations: 1) Freshly prepared 30 percent artificial sea-water; 2) As series 1, with addition of one part/mil. Difco-proteose-peptone; 3) Artificial sea-water (30 per cent) from an *Artemia* culture; 4) As series 3, with the addition of one part/mil. Difco-proteose-peptone; 5) Artificial sea-water (30 per cent) in which bacteria were grown for some time; 6) As series 5, with the addition of one part/mil. Difco-proteose-peptone.

In the preparation of the medium used in series 5 and 6, one part dry yeast per 10,000 parts water was added to the artificial sea-water. The medium was inoculated with a species of bacteria isolated from the enrichment cultures and was kept at 28°C for two weeks. Before using it for the *Prymnesium* cultures, the water was filtered, diluted, and then treated like the solution used in the other experimental series.

The first cultures of this experiment were established by transferring 0.5 ml from the standard cultures. They were continued by serially transferring 0.5 ml from each experimental series into identical nutrient media.

Population maxima reached in the third subculture

Series	*cells per ml*
1	40,000
2	190,000
3	900,000
4	3,000,000
5	960,000
6	2,000,000

The results clearly show two facts:

In the first place, *Prymnesium* grew best in water from *Artemia* cultures (series 3 and 4). Similarly favourable results were also obtained in water from bacterial cultures (series 5 and 6). In fresh sea-water, on the other hand, growth was very weak (series

1 and 2). Additional cultures were set up in fresh sea-water with the addition of yeast, as well as in sea-water in which the same species of bacteria was grown in different substances, among which were the feeds used in the fish ponds. It was apparent that *Prymnesium* grew well in the various media with bacteria. In fresh sea-water, on the other hand, even with the addition of yeast, it grew poorly. These results proved that the bacterium isolated from the enrichment cultures secreted a substance which stimulated the growth of *Prymnesium* (and which is not derived from *Artemia*). This substance is, however, not essential for *Prymnesium*, since development of the flagellates, though weak, did take place in pure, freshly prepared artificial sea-water.

Pringsheim (1952) found that *Ochromonas*, another *Chrysomonadina*, also needed a vitamin source for its development — in his case a liver extract. But *Ochromonas* also grew without liver extract in cultures infected with bacteria. Pringsheim interprets his results as due to the formation of a growth substance by the bacteria.

The second fact shown by the comparison of experimental series 1, 3, and 5 with 2, 4 and 6 was that the addition of Proteose-peptone greatly increased the growth of *Prymnesium* in all the solutions used. Not all peptone preparations acted equally well. In another experimental series different peptone preparations were tried in water from *Artemia* cultures, and the following maximal populations of *Prymnesium* were obtained:

	(*cells/ml*)
Without addition of peptone	390,000
Difco-Yeast extract	320,000
Bacto casamino acids	320,000
Bacto vitamin-free casamino acids	290,000
Bacto tryptone	330,000
Bacto casitone	1,560,000
Proteose peptone	1,860,000
Bacto tryptone	1,120,000
Neo-peptone	1,250,000
Bacto peptone	1,740,000

Two groups could thus be clearly distinguished among the peptone preparations studied: those which had no influence at all on the growth of *Prymnesium* and those which increased growth three to four times in comparison with the peptone-free solution. The action of the peptones of the first group was not at all improved by the addition of yeast extract. Pringsheim (1952) found that *Ochromonas* also needs an organic source of nitrogen. He, too, found that different peptone preparations did not act equally well. But, contrary to our findings on *Prymnesium*, the addition of liver extract increased the influence of peptones otherwise ineffective for *Ochromonas*.

On the basis of these experiments we can explain the mass development of *Prymnesium* in our ponds as caused by an accumulation of feed remnants not used by the fish. These serve, on the one hand, as a substrate for the bacteria which produce growth substance, while on the other hand, they directly enrich the pond water with peptone-like substance. In this connection it should be mentioned that Otterstrom and Steemann-Nielsen (1939) found that the mass-appearance of *Prymnesium* described by them occurred in ponds polluted with organic substances by the sewage from a dairy. This

also explains why the brackish water ponds in the Far East, in which the fish live only on natural food without any addition of feed stuffs, have so far been spared from *Prymnesium* damage.

Pringsheim (1952) found carbohydrate, and particularly glucose, to favour the growth of *Ochronomas*. We therefore studied the influence of the addition of glucose to *Prymnesium* cultures. For this purpose *Prymnesium* was first grown in peptone- and glucose-free medium in two sub-cultures. In the third sub-culture it was transferred to the following experimental series:

1) *Artemia* water plus 1 p./m. proteose peptone plus 1 p./m. glucose; 2) *Artemia* water plus 1 p./m. proteose peptone without glucose; 3) *Artemia* water plus 1 p./m. glucose without peptone; 4) *Artemia* water without peptone and without glucose.

The population maxima obtained were:

Series	*cells/ml*
1	3,050,000
2	3,060,000
3	41,000
4	37,000

This experiment again demonstrates the effect of peptone addition on the growth of *Prymnesium*, and shows that glucose has no effect on growth.

c) *Salt concentrations in which development of* Prymnesium *was possible*

Our field observations showed the appearance of *Prymnesium* to be limited to brackish water ponds. Since there are plans for an extension of fish culture to sea-water ponds as well, it seemed of importance to determine experimentally in what concentrations of salt *Prymnesium* can develop. These experiments were conducted in three different experimental series in which sea-water from *Artemia* cultures at dilutions of 1 : 80, 1 : 40 and 1 : 8 served as the basic solutions. To these solutions various amounts of NaCl were added and in this way the different total salt concentrations were obtained. From these tests it appeared that the absolute concentrations of population obtained, as well as the limits of development of *Prymnesium*, varied with the concentration of the basic solution; the best results being obtained with the 1 : 8 dilution. This is understandable, since with increased dilution of the *Artemia* sea-water the growth factor it contains also becomes diluted. This sea-water concentration is, however, above the lowest limit of the salt concentration necessary for the development of *Prymnesium* and it could be used only in experiments on the determination of the upper limits of the salt concentration.

In summarizing our results we find that we never obtained development of *Prymnesium* at less than 0.9 part/mil. salt content. This may also represent the lowest limit for the appearance of *Prymnesium* under natural conditions. We found weak development between 1.1 and 2.0 part/mil. salt content; good growth in the wide range of 2.5 to 30.0 part/mil.; and a distinct decrease in growth between 30.0 and 45.0 part/mil. In salt concentrations of over 50.0 part/mil., live *Prymnesium* were found only in exceptional cases. These results show that *Prymnesium* is capable of development in undiluted sea water.

d) *Influence of pH on the development of* Prymnesium

In cultures containing bacteria, *Prymnesium* was observed to grow best in solutions with a basic pH. To our surprise we found that, in bacteria-free cultures, growth of *Prymnesium* was also possible in slightly acid nutrient solutions. In general, pH 6.0 may be designated as the lower limit, but in some series growth was still observed at a pH of 5.3. Between pH 6.25 and pH 8.4 growth was independent of the reaction of the media. In this respect, also, *Prymnesium* behaves differently from *Ochromonas* examined by Pringsheim (1952). *Ochromonas* preferred acid solutions and developed best at pH 4 to 6, while at pH 6.4 no further development was observed.

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REVISION OF THE GENUS *HYALOMMA*

I. DESCRIPTION OF KOCH'S TYPES

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During the last 30 years, the genus *Hyalomma* has been subject to considerable controversy. The genus was established in 1844 by Koch, who described 13 species and mentioned 3 others previously described. Neumann (1911) reduced the number of hitherto described species to three with four subspecies, adding one new species. He included most of the species now recognized in *H. aegyptium* and, up to 1930, most authors referred to almost any *Hyalomma* as *H. aegyptium*. On the other hand, Schulze and Schlottke (1927/28) described about 50 species in their key to the genus *Hyalomma*. Later, Schulze and others added about 30 additional new species and subspecies.

Unfortunately Nuttall (1913/14) in his important papers on the biology of ticks refers a tick collected from sheep to *H. aegyptium* L. It is obvious that his work did not deal with *H. aegyptium* as this tick does not attack domestic stock, and without a re-examination of his material it is impossible to state to which species these observations actually refer.

Since 1844, when Koch published his "Systematische Uebersicht ueber die Ordnungen der Zecken", relatively few contributions have been made to the subject. Among these, the following should be mentioned as the most important for the knowledge of the genus.

1) Schulze and Schlottke (1927/28) demonstrated that the tick which Koch (1844) described as *H. syriacum* is the same as *H. aegyptium* L., 1758. This species is known to attack mainly turtles and occasionally hedgehogs and lizards.

2) Delpy (1936) showed the enormous variability in the progeny of one female of *H. dromedarii* and eliminated many species whose description had been based on individual variation, and thus indicated the possibility of wide ranges of variation within other species.

3) Adler and Feldman-Muhsam (1946,1948) confirmed the presence of considerable variations in the offspring of a single female of *Hyalomma*. They showed that most of the characters used by Schulze for specific diagnosis are irrelevant and almost all species and subspecies described by him are not valid because of variability in colour of legs, form of parma and stigma, and minute variations in the punctation of the scutum. They also exploited some new characters not previously used for the specific diagnosis. These characters, such as the female's genital aperture and the peristigmal hairs, are not subject to individual variation (as proved by breeding experiments), and this facilitated the specific differentiation between females of *Hyalomma*, which, according to Delpy (1936) and Kratz (1940), had hitherto been almost impossible.

Kratz, recognizing the fact that the usual method of determination is unsatisfactory and misleading for diagnosis, did not give a key for females. He says "die Bestimmung einzelner ♀ ♀ ist in den meisten Faellen ganz unsicher" (p. 514).

Females were diagnosed by studying the males with which they had been found associated (Delpy 1946, p. 83). As a matter of fact, the only valid character previously used for the diagnosis of the females was the punctation of the scutum which made possible the diagnosis of groups of ticks (e.g. *H. impressum* and *H. rufipes*, with strongly punctated scuta, and *H. excavatum*, *H. dromedarii* and *H. transiens*, with slightly punctated scuta). Delpy (1949), following the work of Adler and Feldman-Muhsam, revised the genus *Hyalomma*, leaving only 10 species. He does not refer to Koch's type material, an examination of which is essential for a sound revision of the genus. The first step in the revision of the genus *Hyalomma* obviously necessitates a detailed description of Koch's types. This omission has been responsible for considerable controversy and superfluous synonymy.

This paper is an attempt to clear up the present confusion on the basis of a re-examination of these types: it contains the description of those of Koch's types which until recently were in Schulze's collection. Unfortunately they had been partly mixed up with other material of Schulze's collection and had not always been kept in such a way that it was possible to ascertain whether they really belonged to Koch's material.

Koch's ticks were pinned and kept in boxes. The labels with the names were also pinned to the bottom of the box but with separate needles, evidently beneath the ticks concerned, as is customary with collections arranged for display. It was not always easy to separate the ticks belonging to different labels. We did not consider a specimen to be part of Koch's original collection without strong supporting evidence. The conditions in which the different type specimens were found will be discussed with respect to each species.

In addition *H. rufipes* will be described. The type of this species has been kindly lent to us by Prof. A. Kaestner, Director of the Zoological Museum of Berlin. Thus all of Koch's types which still exist are covered in the present paper.

As the types are not easily accessible, the following descriptions and the accompanying drawings include some details which, though not necessary for specific diagnosis, are given for the sake of investigators who might be interested.

It should be mentioned that, most unfortunately, some of the investigators who examined Koch's material did not handle these valuable specimens with due care. A comparison of the number of specimens found by Neumann (1901) in Koch's collection with those still existing shows that most of the specimens have disappeared. Others may have been improperly transferred to other species, but records of such transfers could not always be traced. In some instances the little label bearing Koch's name with an asterisk (the mark of Koch's collection) is missing in the original material, and in others the catalogue number is missing. All the handling to which the material has been subjected adds to the difficulties in tracing the original type specimens and in separating them from other material.

As mentioned above, Koch (1844) described 13 species and refers to three additional species previously described by others. The latter three species are: *H. aegyptium* L., *H. forskaolii* Savigny and *H. fabricii* Sav. Among the 13 species described by Koch, two do not belong to the genus *Hyalomma*. According to Robinson (1926) *H. devium* K. = *Amblyomma devium* K. = *A. marmoreum* K., and *H. latum* K. = *Amblyomma latum* K., whereas according to Neumann (1901) the type of *Amblyomma latum* is *Aponomma latum* K.

H. syriacum K. is a synonym to *H. aegyptium* L.

The types of *H. grossum*, *H. anatolicum*, *H. marginatum* and *H. lusitanicum* are lost.

The remaining species, i.e. *H. dromedarii*, *H. impressum*, *H. truncatum*, *H. rufipes*, *H. excavatum*, *H. hispanum*, for which type specimens still exist, are described and discussed in this paper.

In the list of synonyms, an asterisk before the name of the species means that the type specimen has been examined by the present author. Two asterisks before the name of the species indicate that specimens, not types, determined by the very author who had established the species have been examined by the present author. This is not ideal but in the absence of types there is no alternative.

H. DROMEDARII K., 1844

This species is the genotype. Neumann (1901) found in Koch's collection four males and six females. Now there remain only one male and one female labelled *H. dromedarii* K., Type.

The male is almost entirely destroyed by Dermestidae, and only a very thin crust of the scutum remains attached to the capitulum. Nothing remains of the ventral part. The gorged female is partly destroyed and partly broken. As this female had started to oviposit, the capitulum was contracted under the scutum. In order to uncover the basis capituli, the part of the scutum which covered it has been removed. It is therefore clear that in their present condition these male and female specimens can hardly serve any more as types for the species.

Together with these type specimens, the types of *H. dromedarii asiaticum* Sch. and Schl., 1927/28, were found in Schulze's collection. The male type bears in addition to the label "*H. dromedarii asiaticum*" the small label with Koch's name and the asterisk, the mark of Koch's collection in the Zoological Museum of Berlin. There can therefore be no doubt that this specimen has been taken from Koch's material.

The female type of *H. dromedarii asiaticum* Sch. and Schl. bears only the "Type" label (i.e. the label stating that this tick is a type specimen), and it may or may not have been taken from Koch's material. Schulze and Schlottke labelled these ticks *H. dromedarii asiaticum*, and in their key (1927/28) they noted that the species is from Bukhara. Koch (1844) stated that *H. dromedarii* is to be found in Egypt and Asia Minor, but in 1847 he added Bukhara. This shows that he had specimens from Bukhara too and the specimens in question might therefore well be of Koch's material from Bukhara. We nevertheless think that these specimens could not serve as types for *H. dromedarii* because they differ from what is usually called *H. dromedarii*.

The above mentioned types of *H. dromedarii* K. are in bad condition but the poor remains correspond well with what is currently considered *H. dromedarii*. *H. dromedarii* K. will therefore be described on the basis of laboratory-bred material.

MALE

The male scutum has generally a red-brown colour, but may be darker or lighter. The length of the scutum is about 4.5 mm, width ca. 3.2 mm. The scutum is smooth and glossy, with a few large punctations scattered on the anterior half only. The scapulae are covered with many punctations, as is common in the genus *Hyalomma*. The cervical grooves are deep and very long; they extend beyond the middle of the

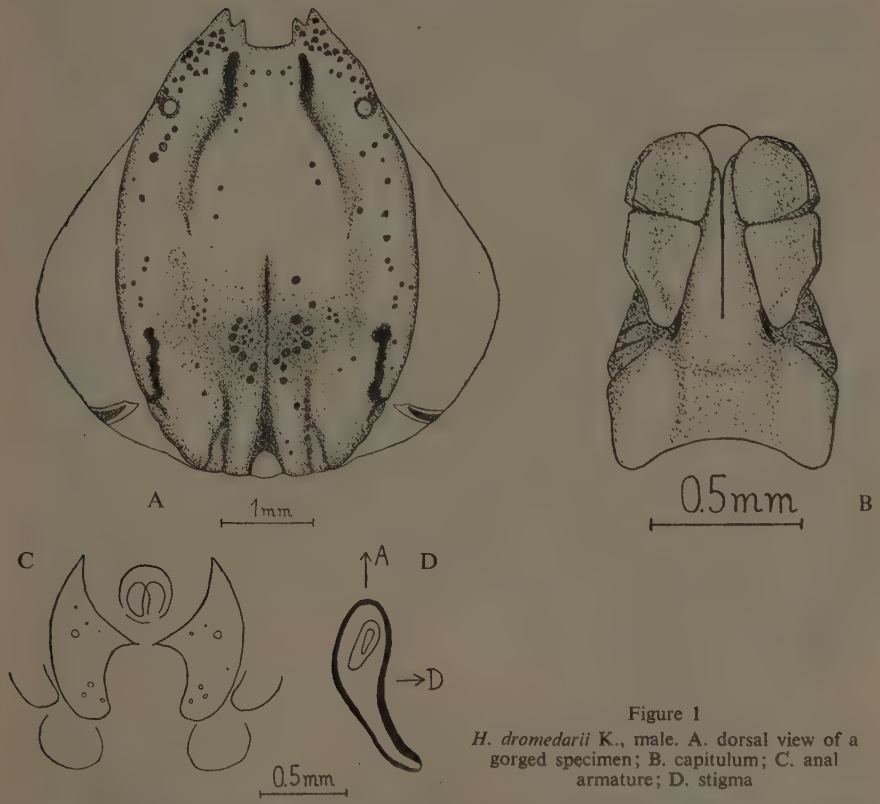


Figure 1
H. dromedarii K., male. A. dorsal view of a gorged specimen; B. capitulum; C. anal armature; D. stigma

scutum. The lateral grooves are very short. There are short paramedian grooves which are the continuation of the marginal notches between the first festoon and the second one and the second and the third festoons. The median groove reaches the parma. The second festoon from the parma is very long. The third, fourth and fifth festoons are fused (Figure 1A). The palps are relatively short. There are two longitudinal swellings on the basis capituli (Figure 1B).

The anal armature is very characteristic for the species. The lateral margin of the anal plate is convex and continues smoothly into the distal margin without forming any lateral posterior angle; the internal margin is concave and forms a very long and acute prolongation towards the opposite plate behind the anus. The adanal plates are rounded distally (Figure 1C). Sometimes there are two subanal plates on each side. In ungorged specimens the subanal plates are partly behind the anal plates and partly behind the adanal plates. In gorged specimens they are completely behind the adanal plates. The stigma is comma shaped (Figure 1D).

The males of *H. dromedarii* become excessively distended laterally when they are gorged. The dilatation takes place in the hinder half of the body, and the tick then becomes pear shaped (Figure 1A).

FEMALE

The ungorged female is ca. 4.6 mm in length. The length of the scutum approximately equals its width; its colours may be brown or yellow-brown. There are a few large punctations scattered on the scutum, the scapulae being densely punctated, as usual. The palps are somewhat shorter than in the other species of the genus. The legs are yellowish.

The best characteristic for specific diagnosis of the female is the form of the cleared and mounted genital aperture (Adler and Feldman-Muhsam 1946, 1948). In some cases it is possible to diagnose the females also by the external form of the unmounted genital aperture (Delpy 1949).

In the *Ixodidae* the female genital aperture consists of a more or less long tube (vagina). The opening of this tube is a chitinized structure which differs in the various species. The entrance to the vaginal tube is in some species entirely bare, in others it is protected by a fold of the external tegument of the tick, which forms a little knob. This knob or operculum may cover the entrance into the vagina, or it may be somewhat anterior to the entrance which is then uncovered. More details of the structure of the upper portion of the genital aperture can be discerned if it is mounted and examined microscopically. The unmounted genital aperture of *H. dromedarii* has the form of a long and deep V. The area within the angle of the V is flat (Figure 2A). When cleared and mounted in balsam, the form of the V is seen with more precision, the flaps being thick (Figure 3).

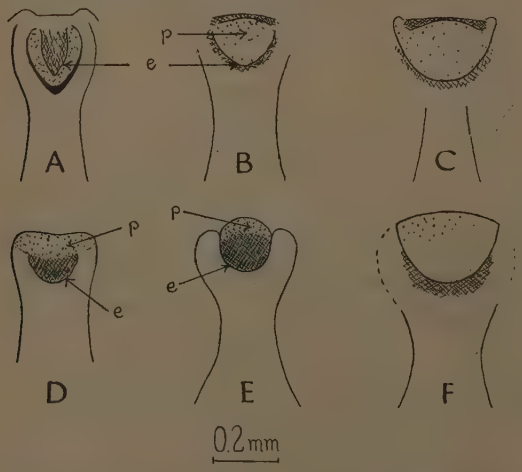


Figure 2

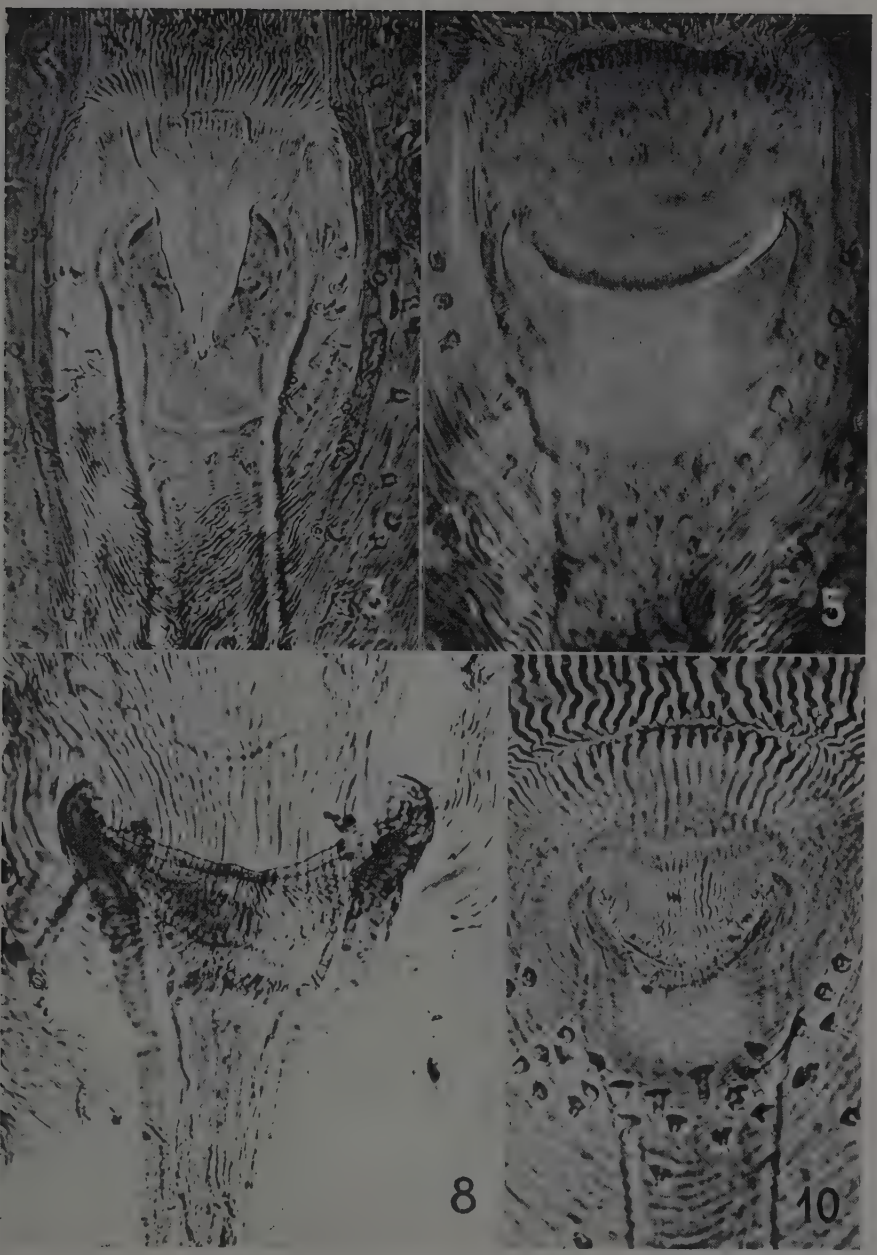
External appearance of the female genital aperture (unmounted tick).

A. *H. dromedarii*
B. *H. excavatum*
C. *H. marginatum*
D. *H. impressum*
E. *H. truncatum*
F. *H. rufipes*

e = opening of the genital tube
p = protruding knob
Dotted area = protruding area
Hatched area = depressed area

DISCUSSION

Schulze, who examined the types of *H. dromedarii* K. probably before they had been damaged, noticed the difference between them and Koch's other specimens which he called *H. dromedarii asiaticum*. The type of *H. dromedarii asiaticum* Sch. and Schl. is thus the only remaining specimen of Koch's material still in good condition which we have been able to trace, but it would not, in our opinion, be justifiable for this reason only to consider this specimen as the type for *H. dromedarii* K. The present con-



Cleared and mounted genital aperture of females (× 200)

Figure 3. *H. dromedarii* K. Figure 5. *H. excavatum* (laboratory bred).

Figure 8. *H. marginatum* K., Type. Figure 10. *H. impressum*, K.

cept of *H. dromedarii* K. is accepted by entomologists and applied to widely distributed and much studied forms for which unfortunately a type specimen in good condition is no longer available. The remains of the type specimen show that its scutum is wide in contrast to the long and narrow scutum of *H. dromedarii asiaticum*. The wide scutum is characteristic of what is currently accepted as *H. dromedarii*. Thus although the remaining, badly damaged type specimen cannot serve as a type specimen, we can not use the other specimen of Koch's collection (the type of *H. dromedarii asiaticum* Sch. and Schl.) as a type for *H. dromedarii*. The concept of *H. dromedarii* accepted by medical entomologists seems to be correct. *H. dromedarii asiaticum* Sch. and Schl. will not be described in the present paper which is concerned with Koch's types only.

SYNONYMY

Schulze created a *dromedarii* group consisting of *H. dromedarii dromedarii* K., 1844, *H. dromedarii asiaticum* Sch. and Schl., 1927/28, *H. dromedarii canariense* Sch. and Schl., 1927/28, *H. asiaticum citripes* Sch., 1935, and *H. delpyi* Sch. and Gossel, 1936. All these were later incorporated by Delpy (1936) into *H. dromedarii*. Kratz accepted Delpy's view but he maintained *H. dromedarii citripes* as a distinct species. This species was created by Schulze in 1935 for specimens from the Karakorum area and called by him *H. asiaticum citripes*. The type specimens of *H. dromedarii asiaticum* as well as *H. asiaticum citripes* differ from *H. dromedarii* and are not synonyms of *H. dromedarii*.

The male type of *H. dromedarii canariense* Sch. and Schl., 1927/28, is a typical *H. dromedarii* with very large subanal plates. The genital aperture of the allotype is typical of *H. dromedarii*.

SYNONYMS

H. aegyptium var. *dromedarii* K., 1844: Neumann, 1901. p.p.
H. aegyptium dromedarii K., 1844: Neumann, 1911. p.p.
**H. dromedarii canariense* Sch. and Schl., 1927/28.
H. yakimovi Olenev, 1931.
H. yakimovi morpha persiacum Oleñev, 1931.
H. delpyi Sch. and Gossel, 1936.

H. EXCAVATUM K., 1844

A single male tick with three labels, one marked "Type", another "*H. excavatum* Koch", and the third "*H. impressum albiparmatum*" Sch., was found in Schulze's collection together with other ticks of Koch's collection. We assume that this tick is Koch's type of *H. excavatum* because:

i) The type specimen of *H. excavatum* is missing from the Zoological Museum of Berlin, although, according to Neumann (1901), there should be one male specimen belonging to this species.

ii) This tick cannot be the type of *H. impressum albiparmatum* (= *H. aegyptium albiparmatum* Sch., 1919), as the types of this species (one male and one female) are other specimens which are present in the Berlin Museum.

iii) It is most improbable that Schulze, after having named a tick, would have added another label mentioning the name by which Koch would have named the same tick.

iv) Koch's description of this species agrees very well with this male specimen.

It seems most probable, therefore, that the type label belongs to *H. excavatum*, and that Schulze took Koch's type of *H. excavatum* and determined it as *H. impressum albiparmatum*.

The female of *H. excavatum* was unknown to Koch, and there is obviously no type specimen. The female will therefore be described on the basis of laboratory bred material.

MALE

The length of the scutum is ca. 4 mm, the width ca. 2.6 mm. The cervical grooves are long and superficial. The lateral grooves are very short. The parma is white. The third, fourth and fifth festoons are fused. At the fused festoons starts a longitudinal elevation which extends anteriorly up to the hinder third of the scutum. Between these elevations, the whole caudal field is strongly depressed. The depressed field is finely punctated (Figure 4A). The outer margin of the anal plates is strongly convex laterally. The inner margin of the anal plates forms an acute extension behind the anus (Figure 4B).

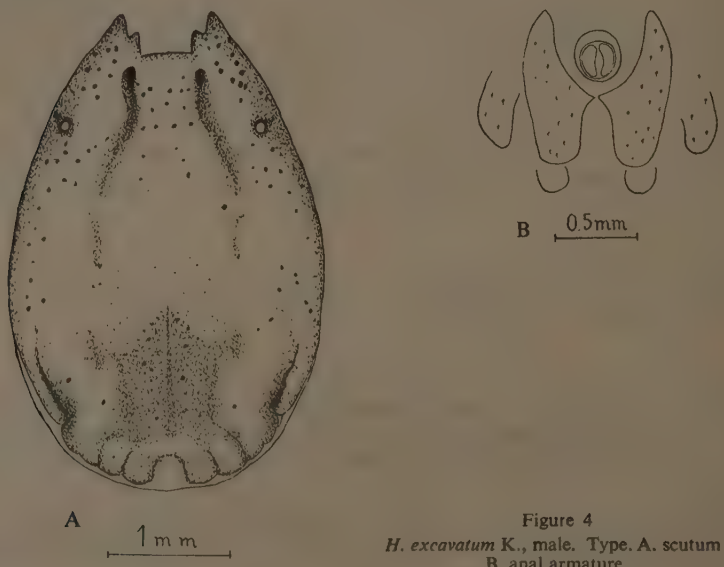


Figure 4
H. excavatum K., male. Type. A. scutum; B. anal armature.

FEMALE

The female of *H. excavatum* is very variable in size and colour. The ungorged female is ca. 4.5 mm in length. The colour of the scutum varies from pale yellow-brown to brown, but it is generally light red-brown. The scutum is longer than broad and slightly punctated with very fine dots and a few larger ones. The larger dots are distributed in the scapulae and on the area between the cervical grooves. The legs are yellow-brown. The form of the unmounted genital aperture is variable. The

operculum has the form of a little knob which may be either round or elongated transversally or longitudinally (Figure 2B). In the mounted genital aperture the upper edge of the vagina is only slightly excavated, and the operculum just reaches the opening of the vagina, or slightly overlaps it (Figure 5).

DISCUSSION

Males differing very slightly from the above described pattern were determined by Schulze as *H. savignyi* Gervais. Following Schulze's key and relying on specimens determined by Schulze, Adler and Feldman-Muhsam (1946, 1948) also called such specimens *H. savignyi*. After examining Koch's type it became evident that *H. savignyi* Gerv., as determined by Schulze, and *H. excavatum* K., are synonyms; and as both names date from 1844, the question arose which of these two names should be maintained.

The paramount advantage of the name *H. excavatum* is that it was given to a specimen which fortunately is still existing, while *Ixodes savignyi* is the name given by Gervais to Savigny's drawing of a tick which Savigny himself had called *Ixodes aegyptius*. In addition, an examination of Savigny's drawing in "Description de l'Egypte" (Pl. 9, Figure 10 and 10.2) or the copy of these drawings by Gervais in Walkenaer, Histoire Naturelle des Insectes Aptères, pl. 32, reveals considerable inaccuracies.

Savigny's Figure 10 shows a female viewed dorsally. The only characteristic of this female is the strongly punctated scutum. Figure 10.2 shows a male specimen viewed ventrally and the caption of this figure says: "Le même individu très grossi, vu en dessous". It is clear that it is not the same specimen.

Our arguments against the use of the name *H. savignyi* for any known species are as follows:

i) Savigny's drawings refer to more than a single specimen.

ii) The punctate scutum of the female cannot be of *H. savignyi* in the sense of Schulze, nor in that of Delpy; in neither species is the scutum so strongly and conspicuously punctated. A scutum like that of the female drawn by Savigny could belong only to *H. impressum* or *H. rufipes*.

iii) The ventral view of the male shows a first coxa deeply divided. The anal armature shows four subanal plates on each side, an arrangement which never occurs in *Hyalomma*.

After having examined Savigny's drawings, we are surprised that Schulze re-established the name *H. savignyi* and related a certain species, or more exactly a group of specimens with a particular pattern of the scutum, to this name. It is also surprising that Delpy (1946), relying on Schulze's revaluation of the species, arbitrarily and without being able to examine a type specimen or consulting a good description (as neither existed), attached this name to another species, namely the one called by Schulze *H. marginatum* K. None of the characters used by Schulze or Delpy for diagnosis can be discerned in Savigny's drawings. We therefore think that the drawings of Savigny could not be related to any known species of *Hyalomma*, and that the name *H. savignyi* should be considered a nomen-nudum.

SYNONYMY

It seems that this species is the most variable among all species of *Hyalomma* and has, in consequence, received many different names. A mere study of the dorsal relief of

the male would induce the investigator to create many different species, as Schulze actually did. But the study of laboratory bred specimens and of the mounted genital aperture of the females reveals the synonymy of many names.

The list of synonyms of this species is probably much larger than that given here: this list includes only names for which synonymy was established on the basis of the examination of types, or specimens determined by the authors who actually created the species, or of descriptions and reproductions which left no doubt as to their identity.

The type of *H. anatolicum* K. seems to be lost. There is no indication in the literature whether Schulze, who used this name, ever saw the type; but specimens of *H. anatolicum* determined by Schulze are within the range of variation of H. *excavatum*.

The male type of *H. pusillum* Sch., 1919, is a small specimen of *H. excavatum* which has subanal plates.

The male type of *H. savignyi exsul* Sch. & Schl., 1927/28, is a typical *H. excavatum* with short lateral grooves, a white parma and a depressed caudal field.

The male type of *H. savignyi iberum* Sch. and Schl., 1927/28, is also a synonym of *H. excavatum* with short lateral grooves, depressed caudal area, and typical subanal plates. Kratz (1940) considers *H. iberum* to be a valid species, characterized by the absence of peltae and by the small size of the main body of the stigma. Examination of the type specimen revealed the presence of peltae and that the stigma did not deviate from the normal. It should be mentioned that Delpy (1949) considers *H. iberum* to be a synonym of *H. savignyi* Gerv. (sensu Delpy), but it seems that this view is not based on an actual examination of the type specimen.

The male type of *H. savignyi mesopotamium* is typical *H. excavatum*. The genital aperture of the allotype is somewhat more excavated than in other specimens. Another male and female of Schulze's collection which we had the opportunity to examine, from Tel-Halef, and determined by him as *H. savignyi mesopotamium*, are typical *H. excavatum*.

The male type of *H. depressum* Sch., 1919 (= *H. lusitanicum depressum* Sch. and Schl., 1927/28), is a characteristic *H. excavatum*. The colour of the scutum of this male varies in different regions from yellow-brown to black-brown. The parma has the same colour as the whole posterior tegument. The female type has the typical genital aperture of *H. excavatum*.

The male type of *H. aegyptium aegyptium* f. *brunnipes* Sch., 1919, is *H. excavatum*. In 1927/28 Schulze and Schlottke transferred *H. aegyptium aegyptium* f. *brunnipes* Sch., 1919, to the *marginatum* group and called the species *H. marginatum balcanicum* f. *brunnipes*. Consequently Delpy (1949) in his list of synonymy considered the species a synonym to *H. marginatum* (*H. savignyi* sensu Delpy). The examination of the male type of this species showed that it is not a *marginatum* at all, but a typical *H. excavatum* with brown scutum, parma and legs, with very short lateral grooves and a depressed caudal field, finely punctated.

The female type of *H. detritum albipictum* f. *ornatipes* Sch., 1919, is an *H. excavatum*.

SYNONYMS

H. aegyptium var. *dromedarii*, K., 1844: Neumann, 1901. p.p.
H. aegyptium dromedarii K., 1844: Neumann, 1911. p.p.
H. aegyptium aegyptium L., 1758: Neumann, 1911. p.p.
H. anatolicum K., 1844.
**H. anatolicum* K., 1844: Schulze and Schl., 1927/28.
H. anatolicum anatolicum K., 1844: Pomeranzev, 1946.
H. anatolicum excavatum K., 1844: Pomeranzev, 1946.
***H. savignyi savignyi* Ger., 1844: Sch. and Schl., 1927/28.
H. savignyi Ger., 1844: Adler and Feldman-Muhsam, 1946, 1948.
H. savignyi Ger., 1844: Feldman-Muhsam, 1947—1952.
**H. savignyi exsul* Sch. and Schl., 1927/28.
**H. savignyi iberum* Sch. and Schl., 1927/28.
**H. iberum* Sch. and Schl., 1927/28: Kratz, 1940.
**H. aegyptium mesopotamium* Sch., 1919.
**H. savignyi mesopotamium* Sch., 1919: Sch. and Schl., 1927/28.
H. savignyi armeniorum Sch. and Schl., 1927/28.
H. armeniorum Sch. and Schl., 1927/28: Kratz, 1940.
**H. pusillum* Sch., 1919.
**H. savignyi pusillum* Sch., 1919: Sch. and Schl., 1927/28.
**H. pusillum alexandrinum* Sch., 1919.
H. lusitanicum K., 1844: Senevet, 1922.
**H. depressum* Sch., 1919.
**H. lusitanicum depressum* Sch., 1919: Sch. and Schl., 1927/28.
H. lusitanicum berberum Sen., 1922.
H. lusitanicum algericum Sen., 1928.
H. lusitanicum cicatricosum Sch. and Schl., 1927/28.
H. tunesiacum Sch. and Schl., 1927/28.
H. turkemeniense Olenev, 1931.
H. tunesiacum turkemeniense Olenev, 1931: Kratz, 1940.
H. tunesiacum franchinii Tonelli-Rondelli, 1932.
H. detritum pavlovskyi Sch. and Schl., 1927/28.
H. tunesiacum pavlovskyi Sch. and Schl., 1927/28: Kratz, 1940.
H. pavlovskyi Sch. and Schl., 1927/28: Olenev, 1931.
H. detritum albipictum f. *ornatipes* Sch., 1919.
H. aegyptium aegyptium f. *excavata*, Sch., 1919.
**H. aegyptium aegyptium* f. *brunnipes* Sch., 1919.
**H. marginatum balcanicum* f. *brunnipes* Sch., 1919: Sch. and Schl., 1927/28.

H. MARGINATUM K., 1844

Among Koch's material in Schulze's collection there were four specimens labelled *H. marginatum hispanum* K. Two of these specimens are females bearing additional labels marked "Type" and "Koch*" (with an asterisk); one male specimen bears three additional labels: the first "Type", the second "1076"+ and the third (in Schulze's handwriting) "Ex. aus Koch's *hispanum*". This male has been determined by Schulze as *H. savignyi* G. The fourth specimen, also a male, is labelled only "Type".

It is clear that the first three specimens are of Koch's material (because of the label "Koch", the number label, and the explicit designation: "Ex. aus Koch's *hispanum*"). Although the fourth specimen is only labelled "Type" and does not bear Koch's name, we conjecture that this specimen is also from Koch's material, because it is the subject of the figures in Kratz's 1940 paper (see Kratz, Figure 33 and the legend). He specifies in his paper that this is Koch's type and that it had been collected in Spain++. In addition, Koch's (1847) description of the male of *H. hispanum* agrees very well with this specimen. Considering the deplorable condition of most of Koch's collection, it seems quite possible that the label "Koch" has been lost subsequent to Kratz's handling. It is therefore highly probable that all four specimens are from Koch's material. It might be mentioned that Neumann (1901) noted that, at that time, there were 2 males and 4 females of *H. hispanum* in Koch's material. In fact, Koch (1844) did not describe a species called *H. marginatum hispanum* but two different species, i.e. *H. marginatum* and *H. hispanum*, but Schulze and Kratz labelled these specimens *H. marginatum hispanum*. Thus the question arises with respect to three of the above mentioned specimens whether they are Koch's *H. hispanum* or *H. marginatum*; with respect to the fourth

+ Dr. W. Crome of the Zoological Museum of Berlin has pointed out that the number 1076 originally belonged to *H. dromedarii* and was apparently attached by mistake to another specimen.

++ Kratz p. 547: In the designation of the type he says: "Type, Koch, 1 ♀, Zool. Museum Berlin". But in the legend to Figure 33 where the male of *H. hispanum* is figured, he says also "♂, Spanien Type" (sic!).

specimen Schulze was fortunately careful enough to state from which species it had been taken. Beside the fact that Koch (1844 and 1847) himself describes only the male of *H. marginatum*, the female being unknown to him, Neumann (1901), examining Koch's collection in Berlin, did not mention *H. marginatum* at all, and Kratz (1940) stated that the type of *H. marginatum* did not exist any more. It is therefore evident that the type specimen of *H. marginatum* was not in the Berlin Museum as early as 1901, and so far we have not been able to find out whether it had been there previously or to trace the collection in which it was originally placed; there is even some possibility that it had already been lost before Neumann's time. There can therefore be no doubt that all the four specimens in question can only be *H. hispanum* of Koch's material.

The examination of the two males shows that they belong to two different species. One (No. 1076), as already noted, was determined by Schulze as *H. savignyi* Gerv. (=*H. excavatum* K.). The other male is in our opinion *H. marginatum* K. (Kratz, Figure 33, and our Figure 6A). Schulze and Kratz already noticed the resemblance of this specimen to *H. marginatum* and determined it as a subspecies of *H. marginatum*. Kratz (1940, p. 545) writes: "Das von Koch als selbstaendige Art aufgestellte *Hyalomma hispanum* gehoert als *H. marginatum hispanum* in diese Gruppe".

The two females also differ. One female is a little smaller than the other. The smaller specimen belongs to *H. excavatum*; the other female, which is somewhat larger, is *H. marginatum*.

MALE

The length of the scutum is ca. 4 mm. The width is ca. 2.5 mm. The colour is red-brown. It should be noted that the colour of most specimens of this species is much darker; but there is a wide range of variation of colour of the scutum within the species: in laboratory breedings of *H. marginatum*, the colour of the scutum in the offspring of one female varied from pale yellow-brown through red-brown to dark brown. The

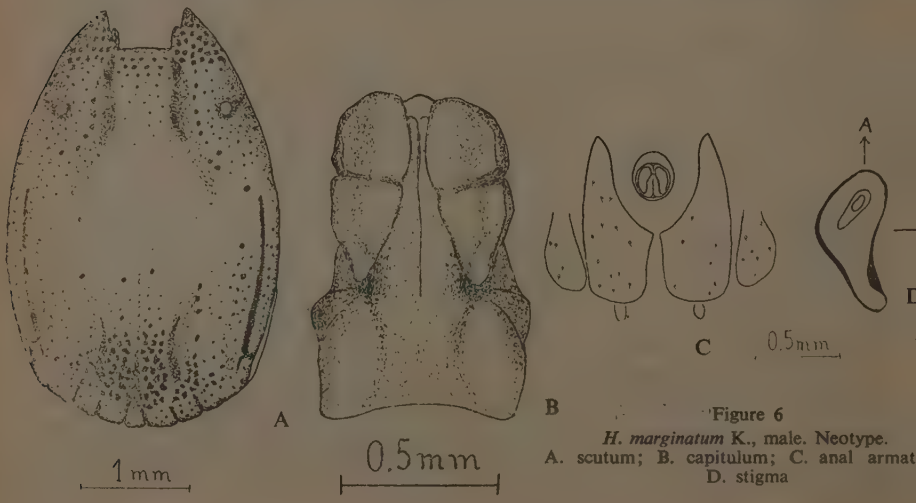


Figure 6
H. marginatum K., male. Neotype.
A. scutum; B. capitulum; C. anal armat
D. stigma

cervical grooves are long; the lateral grooves are long. There are short paramedian grooves which are very characteristic in this species (Figure 6A). The parma has the same colour as the whole scutum. The scapulae and caudal area are covered with large punctations. The anal plates do not form acute angles at their point of meeting behind the anus, and are quite large at their distal end (in contrast to *H. excavatum*) (Figure 6C).

FEMALE

The length of the scutum is ca. 2.6 mm and is equal to the width. The area between the cervical grooves is red-brown. The area outside the cervical grooves is black-brown. The punctations of the scutum of this female are shallow and are limited to the anterior half (Figure 7A). This is the appearance in most of the females of *H. marginatum* (the punctation might be even less prominent). This point should be particularly stressed as it stands in contrast to the drawing of *H. savignyi* Ger. in Description de l'Egypte, which Delpy considers to be the type for that species. As already noted in connection with *H. excavatum* (see our arguments p. 132), the drawing of the female of *H. savignyi* Ger. shows a very punctate scutum. *H. marginatum* (*H. savignyi* sensu Delpy) never has such a punctate scutum.

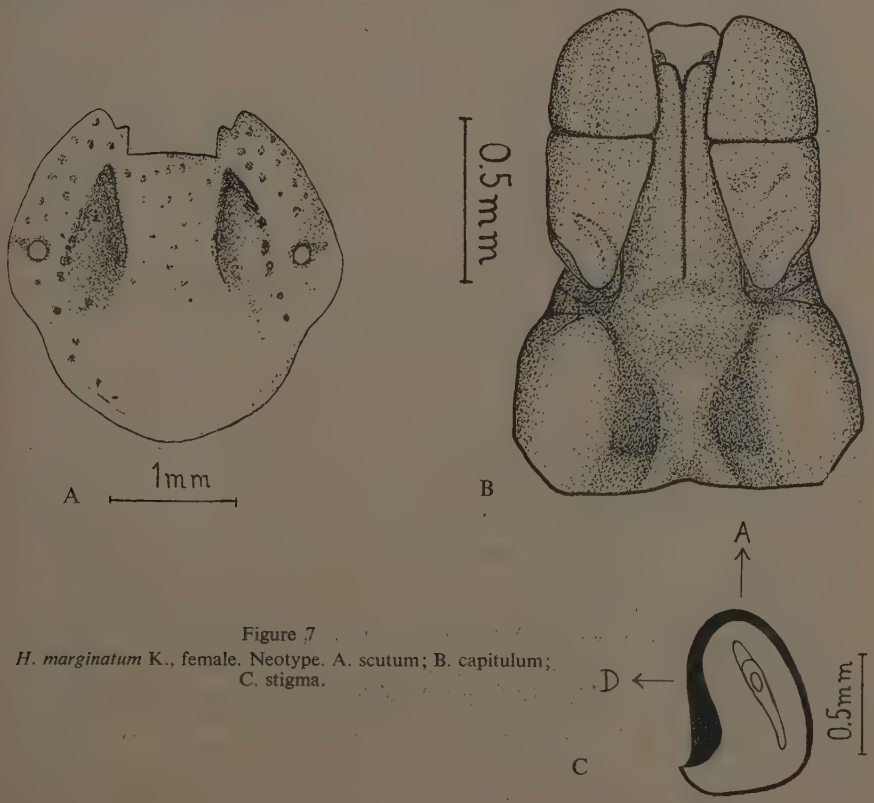


Figure 7
H. marginatum K., female. Neotype. A. scutum; B. capitulum; C. stigma.

The width of the basis capituli is about 1.5 times its length (Figure 7B). The form of the female's genital aperture is the only reliable character for the diagnosis of the female.

The operculum of the genital aperture is big, highly protruding, and somewhat wider than long (Figure 2C). When microscopically studied, the upper edge of the opening of the genital tube appears very shallow. The lateral flaps are more chitinized than the underlying tube. The operculum overlaps beyond the opening of the genital tube (Figure 8).

Another good characteristic for the female (also for the male!) of this species is the number of "tunnels" in the anterior portion of the scutum (Adler and Feldman-Muhsam 1946, 1948). But as this specimen could not, for obvious reasons, be mounted, we are unable to state the number of "tunnels" in this specimen.

DISCUSSION

According to Koch, the species had been described by Fabricius (1794) under the name *Acarus hispanus* (Koch 1844 and 1847). Kratz cites Schulze's opinion that Fabricius probably described a *Rhipicephalus*, because the description says: "Antennae breves, extrorsum crassiores". For Delpy, *Acarus hispanus* is a synonym of *H. savignyi* Ger. and would have had the priority, if Fabricius's description had not been so vague. In our opinion it is more probable that Fabricius had a *Hyalomma* before him rather than a *Rhipicephalus*, because Fabricius's description says also "geniculis albis" which is most characteristic for *Hyalomma*. The description "antennae breves" might be applied to *Hyalomma*, when one compares the palpes of *Hyalomma* with those of *Amblyomma* or *Ixodes*. But there is no doubt that it is impossible to relate any known species to Fabricius' description.

Koch's description of *H. hispanum* agrees very well with one male (Figure 6A) which is also the subject of Kratz's drawing of *H. marginatum hispanum* (Kratz's Figure 33).

As it seems that *H. hispanum* is the same as *H. marginatum*, the question arises whether the species should be called *H. hispanum* or *H. marginatum* or *H. savignyi* according to Delpy. As a justification for the use of the name *H. savignyi*, Delpy (1949, p. 480) says that this name is as good as any other, and as Schulze and Schlottke (1927/28) had revaluated it, he also accepted it. But Delpy designates by this name a species which Schulze calls *H. marginatum* Koch. Delpy uses the name *H. savignyi*, also because he considers that some of Schulze's subspecies of *H. savignyi*, e. g. *H. savignyi exsul* and *H. savigny iberum*, are synonyms of *H. savignyi* sensu Delpy. But by examining the types of H. *savignyi exsul* and *H. savignyi iberum* we could convince ourselves that they are synonyms of *H. savignyi* sensu Schulze. Delpy adds also in the same context that ticks which he considers as *H. savignyi* are described by Koch under the name of *H. marginatum* and *H. hispanum*.

In our opinion, it is not permissible to call this species *H. savignyi*, for the same reasons as those for which we rejected this name for *H. excavatum* (= *H. savignyi* sensu Schulze). There remains therefore the choice between Koch's two names. On the one hand, the type of *H. marginatum* is lost and Koch's description of this species is vague; at the same time the types of *H. hispanum* are existent and Koch's description enables to recognize the species. On the other hand, it seems that *H. hispanum* is reserved by Fabricius for another species, and according to the laws of nomenclature

the species in question should receive another name. As the name *H. marginatum* is extensively used by European and Russian authors, we suggest to retain this name as a nomen conservandum. We also suggest that the types of *H. hispanum* (1 ♂ + 1 ♀) should serve as neotypes for *H. marginatum*.

SYNONYMY

We have had the opportunity of examining males and females determined by Schulze as *H. marginatum brionicum* from Brioni and *H. marginatum balcanicum* from Macedonia. All these specimens do not differ from typical *H. marginatum*.

SYNONYMS

**H. hispanum* Fab., 1794: Koch, 1844.
H. aegyptium aegyptium L., 1758: Neumann, 1911, p. p.
H. aegyptium marginatum K., 1844: Sch., 1919.
***H. marginatum brionicum* Sch. and Schl., 1927/28.
**H. marginatum hispanum* Sch. and Schl., 1927/28.
H. marginatum olenevi Sch. and Schl., 1927/28.
***H. marginatum balcanicum* Sch. and Schl., 1927/28.
H. marginatum bacuense Sch.: Olenev, 1931.
(As far as we know, Schulze never gave this name to any species).
H. marginatum caspium Sch. (Described for the first time by Kratz, 1940).
H. savignyi Gervais, 1844: Delpy, 1946, 1949.

H. IMPRESSUM K., 1844

Only one male of *H. impressum* "No. 1071" exists at present from Koch's material. Neumann(1901), examining Koch's collection, found in addition two females, but they are no longer available. This male type had been in Schulze's collection for many years, and in the same box there were also two females, one of them labelled "*impressum*", and the other unlabelled, but there is no evidence whatsoever that these females originate from Koch's collection. In addition the females are not *H. impressum*. The description of what we consider to be the female of *H. impressum* will be made here on the basis of unidentified material received from the Rocky Mountain Laboratory and collected in Khartoum quarantine on cattle from Darfour (Anglo-Egyptian Sudan). In the same batch there were also *H. rufipes*, *H. truncatum* and *H. impeltatum*.

MALE

The scutum is very typical and easily distinguished from that of other species by a distinctive character, already mentioned by Koch: "Der Hinterleib hinten an den Seiten eingedrueckt". The whole scutum is covered with deep punctations. In the caudal area the punctations are deep, large, and sometimes continuous. The third, fourth and fifth festoons are fused. Cervical grooves short; lateral grooves short (Figure 9A). The colour of the scutum is dark red-brown. The length of the scutum from the middle of the line joining the scapulae to the posterior end ca. 3.7 mm, width 2.4 mm. The stigma is comma shaped, the tail is not very long and it reaches almost to the posterior end of the scutum; the anal plates form acute angles at their point of meeting behind the anus. The adanal plates are big (Figure 9C).

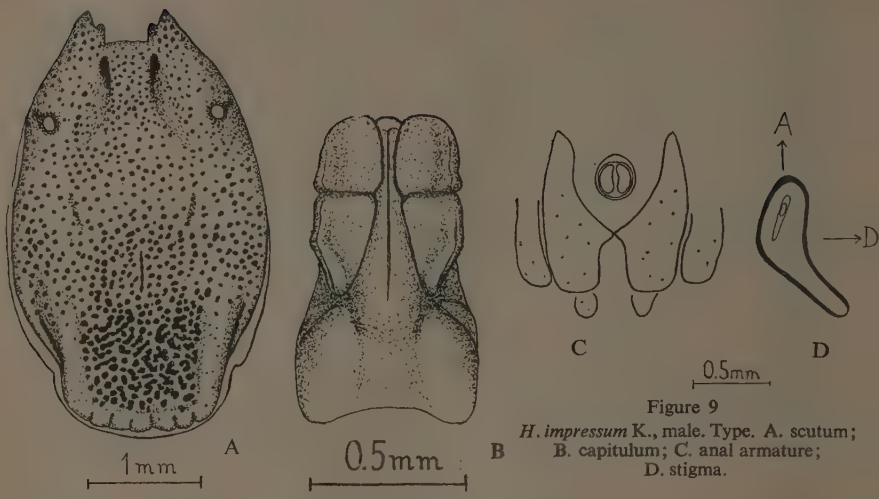


Figure 9
H. impressum K., male. Type. A. scutum; B. capitulum; C. anal armature; D. stigma.

FEMALE

The colour of the scutum is red-brown to dark brown. The whole scutum is covered with deep punctations equally distributed as in the male. The length of the scutum equals its width. The colour of the alloscutum varies from yellow-brown to dark red-brown.

The legs are red-brown with large yellow stripes at the articulations. The unmounted external apron is similar to that of *H. truncatum* (= *H. transiens*). There is a transversally elongated protruding knob (Figure 2D). The area under the knob is excavated, and the entrance into the genital tube is bare. In the mounted genital aperture, the knob appears as a fold of the tegument and the entrance into the vagina is deeply excavated and wide, deeper than in *H. excavatum* (Figure 10).

SYNONYMY

This species is sometimes confused with *H. rufipes* (Delpy 1946; Adler and Feldman-Muhsam 1948) because both species have a dark and strongly punctate scutum, but *H. impressum* is easily distinguished by the posterior lateral constriction of the scutum. The area around the stigma of this species is bare, whereas in *H. rufipes* it is pilose (Adler and Feldman-Muhsam 1946, 1948).

SYNONYMS

H. aegyptium var. *impressum* K., 1844: Neumann, 1901.
H. aegyptium impressum K.: Neumann, 1911.
H. aegyptium impressum f. *typica* K.: Schulze, 1919.
H. impressum impressum K.: Sch. and Schl., 1927/28.
H. impressum K.: Delpy, 1946 p. p.

H. TRUNCATUM K., 1844

H. truncatum is represented by one male tick from Senegal with Catalogue No. 1072 (of the Berlin Museum). Neumann (1901) also mentions only one male of this species in Koch's collection.

As there is no female type of this species the female will be described here from laboratory bred material, kindly sent by Dr. G. Theiler.

Figure 12
H. truncatum, K., female. Cleared and mounted genital aperture (× 200)

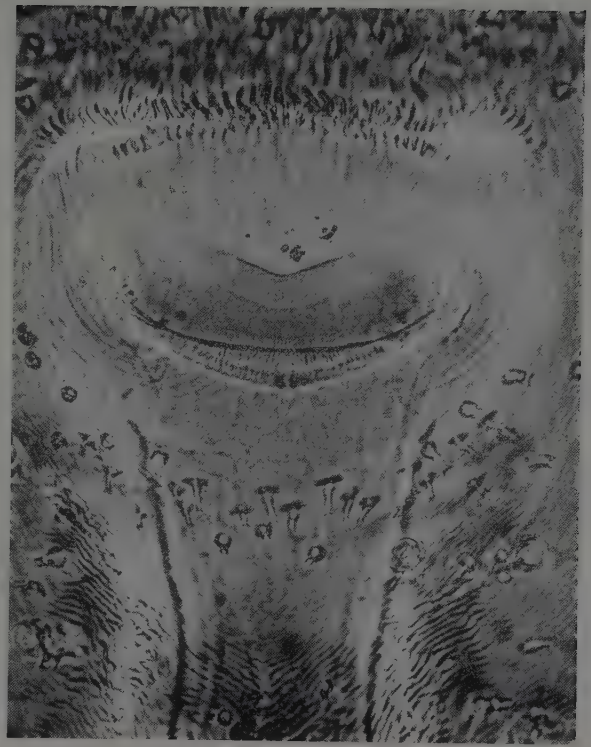


Figure 15
H. rufipes K., female. Cleared and mounted genital aperture (× 200)

MALE

The colour of the scutum is reddish brown (Koch (1844) described it as "reddish black"). Length of the scutum ca. 3.2 mm, width ca. 2.2 mm, cervical grooves very short; lateral grooves very long, almost reaching the eyes. The third, fourth and fifth festoons are fused. The parma has the same shape as the other festoons. The caudal area is punctated with very large contiguous dots. The caudal area is not depressed. Almost the entire scutum besides the caudal area is smooth and glossy and almost without punctations (Figure 11A). The scutum becomes much constricted at the level of the stigmata, almost as in *H. impressum*, but the posterior end of the scu-

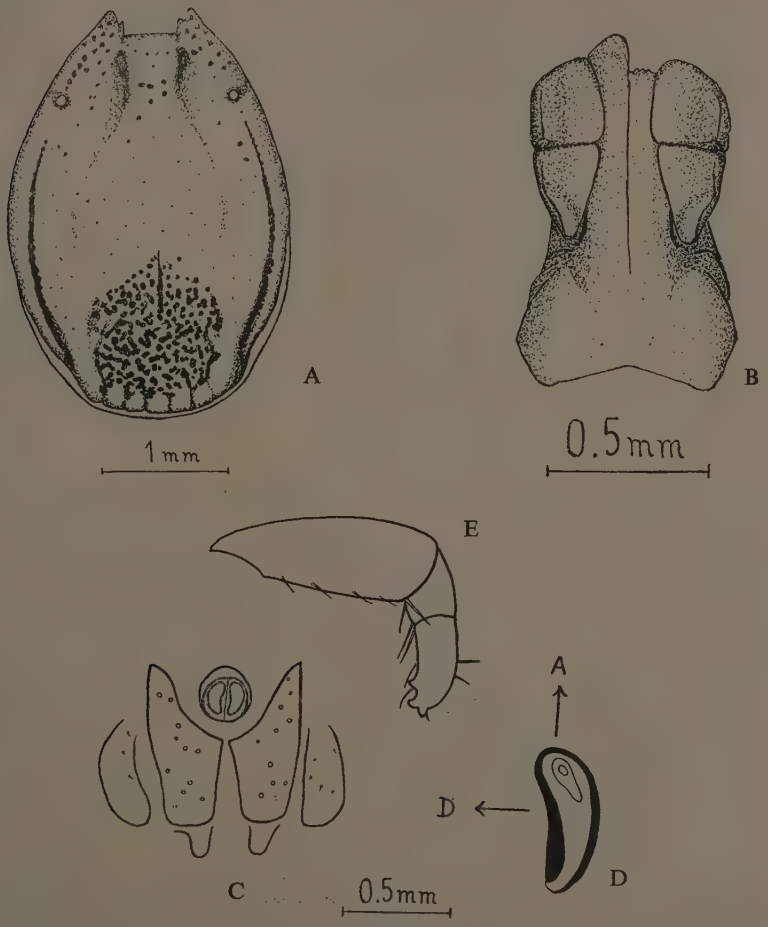


Figure 11

H. truncatum K., male. Type. A. scutum; B. capitulum; C. anal armature; D. stigma; E. IVth tarsus.

tum is rounded and does not form a straight line as in the type of *H. impressum*. The anal plates do not form any prolonged protrusions behind the anus. The adanal plates are large (Figure 11C). The stigma is comma shaped, with a wide tail (Figure 11D).

FEMALE

The colour of the scutum, generally red-brown, varies from yellow-brown to dark, almost black, brown. The punctation is generally fine but some specimens show a coarse punctation.

In the unmounted genital area there is a small transversally elongated protruding knob which does not cover the entrance into the genital tube, and is considerably anterior to it. The entrance to the genital tube is entirely uncovered and appears as half a circle (Figure 2E). Within this half circle the tegument is depressed, whereas in *H. excavatum* the tegument protrudes in this area. The mounted genital aperture shows a well rounded and excavated opening of the vagina (Figure 12).

DISCUSSION

The contour of the scutum of the male is very much like that of *H. impressum* and Koch (1844) already noted the similarity between the two species. The punctation of the scutum of *H. truncatum* is however different and beyond the range of individual variation, and there is no doubt that this specimen belongs to a species quite distinct from *H. impressum*.

The form of the scutum of the male with its grooves and punctation shows very large variation in laboratory bred material. Some males might be confused with *H. marginatum*. The female has no characters which allow its identification apart from the genital aperture. The form of the genital aperture is very characteristic and leaves no doubt as to the specific diagnosis.

SYNONYMY

H. truncatum, which is represented by a single specimen, closely resembles the type of *H. transiens* Sch., 1919. Both have a smooth and glossy scutum with heavily punctated caudal area. Both have five central separated festoons with the third, fourth and fifth lateral festoons fused, very short cervical grooves and very long lateral grooves. Both have a comma shaped stigma with a large tail. The anal plates are very similar; they do not form long and pointed protrusions behind the anus. We could discern only one difference between the types of these two species: this difference concerns the contour of the scutum which is more rounded in *H. truncatum* than in *H. transiens*, laterally as well as posteriorly. In *H. transiens* the scutum is more elongated and the posterior end more rectilinear than in *H. truncatum*.

The examination of laboratory bred material of *H. transiens* kindly put at our disposal by Dr. Gertrud Theiler, showed an enormous range of variation between the offspring of one female. Although at the first glance one would hesitate to decide whether the two specimens (the types of *H. truncatum* and *H. transiens*) belong to the same species or to two different ones, the range of variation observed among laboratory bred material of *H. transiens* shows even greater differences than those existing between the above two specimens. We therefore consider *H. transiens* Sch. as a synonym of *H. truncatum* K.

H. impressum nitidum from Cameroon appears in Schulze's key in 1919 as *H. nitidum*. The male type resembles very much the type of *H. transiens*. The female genital aperture is the same as in *H. truncatum* (= *H. transiens*). Three males from Garua which we have examined, differ from the male type of *H. transiens* only in their very dark colour. Two females from the same lot show the same genital aperture as *H. truncatum*.

H. impressum planum Sch., 1919, from East Africa is probably a synonym of *H. truncatum*. The male type is very similar to the type of *H. transiens*. The genital aperture of the allotype is macroscopically the same as in *H. truncatum*. Microscopically it differs slightly, the lateral flaps being slimmer. Whether or not this difference is within the range of individual variation of the species is still to be studied by additional laboratory breedings.

H. impressum albiparmatum Sch., 1919 (=*H. aegyptium albiparmatum*), from East Africa is very probably also a synonym of *H. truncatum*. The male type (which is present in the Berlin Museum) has an almost smooth scutum with a big white parma. The lateral grooves are long and almost reach the eyes. The genital aperture of the female type (also present in the Berlin Museum) is the same as in *H. truncatum*. In a laboratory bred progeny of one female, kindly sent by Miss J. B. Walker of the East African Veterinary Research Organization, 49 of the males have a big white parma, three males have a small, badly defined white-brown parma, and only one male has a small, brown, indistinct parma as in breedings of typical *H. truncatum*. The genital aperture of the females of this progeny is macroscopically as well as microscopically (when mounted) typical *H. truncatum*.

The examination of two females collected from a horse at Cerniste on 18.6.1917 and determined by Schulze as *H. impressum albiparmatum* showed that one of them is *H. excavatum* and the other *H. truncatum* (= *H. transiens*).

The types of *H. impressum brunneiparmatum* Sch. and Schl., 1927/28, from Togo resemble very much the types of *H. aegyptium albiparmatum*. The only difference is the colour of the parma of the male, which is slightly darker. Such males have been found together with males with big white parma in the laboratory bred progeny, sent to us, as mentioned above, by Miss J. B. Walker. It is highly probable that this name is a synonym of *H. truncatum*.

The ticks determined by Schulze as *Hyalommina lewisi* are not *Hyalommina* but one of the variations encountered in *H. truncatum*.

SYNONYMS

**H. aegyptium aegyptium* L., 1758: Neumann, 1911. p.p.
**H. nitidum* Sch., 1919.
**H. impressum nitidum* Sch., 1919: Sch. and Schl., 1927/28.
**H. planum* Sch., 1919.
**H. impressum planum* Sch., 1919: Sch. and Schl., 1927/28.
?**H. aegyptium albiparmatum* Sch., 1919.
?**H. impressum albiparmatum* Sch., 1919: Sch. and Schl, 1927/28.
**H. aegyptium albiparmatum* f. *transiens* Sch., 1919.
**H. impressum transiens* Sch., 1919 · Sch. and Schl., 1927/28.
H. transiens Sch., 1919. Delpy, 1949.
?**H. impressum brunneiparmatum* Sch. and Schl., 1927/28.
**H. impressum impressum* K., 1844: Kratz, 1940. p. p.
***Hyalommina lewisi* Sch., 1936.

H. RUFIPES K., 1844

In the Zoological Museum, Berlin, there are two males and one females of this species, but only one male bears the label "Type" and another label marked "1073" (the catalogue number). Neumann (1901) also found two males and one female in Koch's collection, all

of which he considered to belong to Koch's material. It is most probable that all three ticks are of Koch's material, because the catalogue number is always attached to one tick only of a group and refers to the group as a whole. But there is no conclusive evidence that all three specimens really belong to Koch's material; we therefore consider the male specimen with the type label as the lectotype, and shall describe this specimen.

The female which was pinned near the male type might or might not belong to Koch's collection; but it definitely does not belong to *H. rufipes*, but to *H. truncatum*. The female of this species will therefore be described on the basis of laboratory bred material, kindly put at our disposal by Dr. G. Theiler.

MALE

The colour of the scutum is dark red-brown. Its length is ca. 4.5 mm and the width is ca. 3.1 mm. It has an elliptical form and is not constricted as in *H. impressum*. It

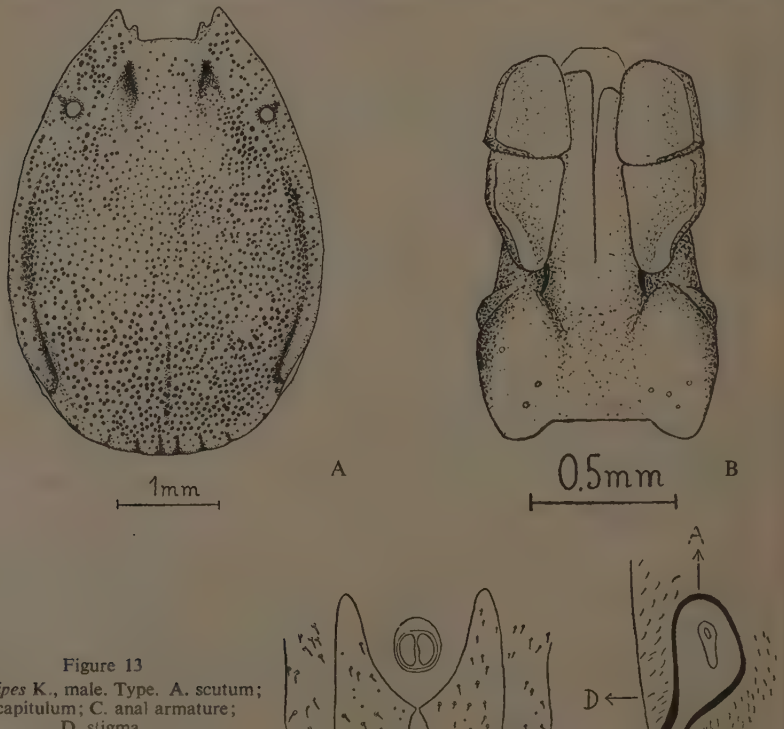


Figure 13
H. rufipes K., male. Type. A. scutum; B. capitulum; C. anal armature; D. stigma.

is covered with large punctations. There are five central festoons; the third, fourth and fifth lateral festoons are fused. The parma is brown. The lateral grooves reach about the hinder third of the scutum, but are continued more anteriorly by large and dense dots which touch each other. The cervical grooves are short and reach somewhat behind the level of the eyes (Figure 13A). The ventral tegument, especially between the coxae, is covered with numerous short hairs. Stigma is retort shaped, with a narrow tail. The tail of the stigma is generally longer than in the type specimen (Figure 13D). The area around the stigma is very pilose (Adler and Feldman-Muhsam 1946, 1948). The anal plates are large and wide. The adanal plates are large, too (Figure 13C).

FEMALE

The colour of the scutum is generally red-brown but may vary from light brown to dark black-brown. The punctations of the scutum are not always as large as those of the male, but it should be noted that in the corresponding area of the male scutum the dots are also relatively small. The colour of the alloscutum and the ventral tegument varies from plain brown to very dark brown, almost black. The legs and capitulum are brown. The area around the stigma is very pilose as in the male. The chitinous spot is also pilose (Figure 14). The operculum of the genital aperture has the form

Figure 14
H. rufipes K., female. Stigma.

of a protruding knob, somewhat wider than long. This knob is similar to that of *H. marginatum*, but is somewhat larger. In the cleared and mounted genital aperture, the upper edge of the vagina is almost flat. The operculum overlaps slightly beyond the entrance into the genital tube. The lower half of the operculum is characteristically thickened.

SYNONYMS

H. aegyptium aegyptium L. 1758: Neumann, 1911. p. p.
H. aegyptium impressum f. *rufipes* K., 1844: Sch., 1919.
H. impressum rufipes K.: Sch. and Schl., 1927/28.
H. aequipunctatum Olenev, 1931.
H. impressum K.: Delpy, 1946. p. p.
H. impressum K., 1844: Adler and Feldman-Muhsam, 1946, 1948.
H. marginatum impressum K., 1844: Pomeranzev, 1946.

H. LUSITANICUM K., 1844

Associated with a label "*H. lusitanicum*" we found in addition to several ticks a pin, bearing a label marked "Type" and an additional label marked "1070", but without

a tick. The last mentioned label indicates material from Koch's collection. The tick was eventually lost or eaten by Dermestidae. The tick had apparently been lost already before 1940, as Kratz does not mention the whereabouts of the type. The type of *H. lusitanicum* should therefore be considered as definitely lost.

Koch (1847) says regarding this species: "In der Gestalt mit *H. hispanum* fast uebereinstimmend". Koch's description of the species is vague and in the absence of material it is difficult to judge whether this was a good species or not. No opinion can therefore be given as to its synonymy.

ACKNOWLEDGEMENTS

I am much indebted to Prof. A. Kaestner and Dr. W. Crome from the Zoologisches Museum, Berlin, for the loan of *H. rufipes* and some of Schulze's types as well as for their patience in responding to continued demands.

I am obliged to Dr. Gertrud Theiler of the South African Veterinary Services and Miss J. B. Walker of the East African Veterinary Research Organization for the loan of material.

I wish to thank Professor S. Adler for advice and criticism and Professor G. Wittenberg for reading the manuscript.

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AN ONION AND TOMATO DISEASE CAUSED BY A VARIETY OF *PSEUDOMONAS SYRINGAE* *

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INTRODUCTION

A bacterial disease on onion leaves and on tomato fruits was found for the first time in Israel at the end of February and at the beginning of April 1953, respectively. Both diseases were caused by the same organism, identified below as a variety of *Pseudomonas syringae* Van Hall.

The incidence of the diseases was limited to one place in the vicinity of Rehovot.

This paper describes the diseases and the causal organism; it also reports investigations to test and prove the pathogenicity of both isolates on onion, tomato, several related hosts, and citrus fruits.

DESCRIPTION OF THE DISEASES

The onion leaf lesions appeared as water soaked transparent soft spots 10—20 mm in diameter.

The tomato fruit lesions appeared as small, brown, soft spots, very slightly sunken, 3—4 mm in diameter.

THE ORGANISM

Isolation

From the lesions of both hosts, a yellow organism was isolated in pure culture on potato glucose and nutrient agar plates at 25°C; it caused lesions similar to those on the original specimens when inoculated on onion leaves and tomato fruits. Inoculation of the onion organism on tomato fruits produced lesions similar to those which were produced by the tomato organism. The same occurred for the tomato organism when inoculated on onion leaves.

Examination of both organisms and of their reisolations has proved that they are identical.

Description

Several types of colonies labelled A, B, C and D were observed on nutrient agar + 1% glycerol plates. Type A was the most common, and was isolated from the lesions of the original specimens on nutrient and potato glucose agar plates. The other types appeared several months later in streaked and poured plates made from the original cultures, and from cultures reisolated from inoculated specimens.

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In successive subcultures of each of the 4, types colonies were usually of the same type as the parent, though one or another of the other 3 types did occasionally appear, but in exceedingly small numbers. In cultures reisolated from lesions of specimens inoculated with a culture of each type, a mixture of the 4 types developed in poured glycerol plates. The majority of colonies, however, were of the same type as that inoculated into the host.

No definite differences were observed in the physiological and morphological characteristics among the four types of colony.

48 hours old culture grown on nutrient agar at 25°C, Gram stain: short rods 0.3 to 0.6 by 1.6 to 2.4 microns, occurring singly and in pairs, Gram negative. Motile, one to several polar flagella. Aerobe and facultative anaerobe. Optimum growth at 23—25°C, maximum temperature 38°C, minimum temperature 5—6°C. Thermal death point approx. 52—53°C. Green fluorescent pigment produced on certain media.

Description of the colonies

Nutrient agar colonies:

Type A, 48 hours old. Circular, smooth colonies, convex, granular, margins entire, light yellow, viscous, 1.8—2 mm in diameter.

Nutrient agar + 1% glycerol colonies:

Type A, 72 hours old. Circular, smooth colonies, glistening, jelly-like, convex, granular, margins slightly ondulate, light yellow, viscous, 3.0—3.5 mm in diameter; bright thin ring appears around the colony when looked at from the back of the plate.

Type B, 72 hours old. Circular colonies, a round small centre surrounded by a round raised thick ring, granular, margins slightly ondulate, deep yellow, very viscous, 3.0—3.5 mm in diameter; bright thin ring appears around the colony when looked at from the back of the plate.

Type C, 72 hours old. Circular colonies, like thick jelly, convex, wrinkled, granular, margin slightly ondulate, deep yellow, very viscous, 3.0—3.5 mm in diameter; bright thin ring appears around the colony when looked at from the back of the plate.

Type D, 48—72 hours old. Circular, smooth colonies, glistening, watery, slightly convex, granular, flat rind, margins ondulate, pale yellow, 2.5—3 mm in diameter.

Nutrient agar + 1% glycerol agar slant:

Original cultures isolated from the lesions of original hosts — profuse growth, yellow, jelly-like, glistening, or wrinkled at first, then turn to a jelly-like, glistening growth, viscous.

Type A. On mosts slants a profuse jelly-like, smooth, glistening, viscous, yellow growth appears. A few cultures appear partly wrinkled, and turn later to jelly-like, smooth, glistening growth.

Type B. Profuse growth, wrinkled, deep yellow, glistening, viscous.

Type C. Profuse growth, wrinkled, deep yellow, glistening, viscous.

Type D. Slightly raised growth, glistening, pale yellow.

Broth slightly turbid, thin gray pellicle formed 48 hours after inoculation.

Gelatin—liquefaction within 3—4 days; starch not hydrolysed; H_2S not formed; indol not formed; nitrate not reduced to nitrite. Milk turns greenish yellow, digested, no

curd; Uschinsky—good growth, green fluorescence; purple lactose—alkaline reaction; pectate gel—alkaline reaction, no liquefaction.

Sensitivity to NaCl — good growth at 5%, slight growth at 7% NaCl.

Carbohydrates fermentation. Acid but no gas produced in glucose, sucrose, maltose, galactose, glycerol, xylose and sorbitol. Lactose and salicin not fermented.

According to the above morphological and physiological data, the organism belongs to the family *Pseudomonadaceae* Winslow et al., tribe *Pseudomonadeae* Kluyver and Van Niel, genus *Pseudomonas* Migula. In view of its close relationship to *Ps. syringae* as well as similar pathological characteristics (described below), the organism was identified as closely related to *Pseudomonas syringae* Van Hall. On account of slight differences in growth characteristics (yellow instead of grey-white, tolerance to 7% NaCl) and especially in view of differences in some pathological characteristics, the organism might be considered a variety of *Pseudomonas syringae* Van Hall (Breed et al. 1948).

INFECTION EXPERIMENTS

Methods and material

Inoculations with the tomato and onion isolates were made on the following hosts: onion leaves; green tomato and pepper fruits (attached and detached); tomato, potato, bean and pepper leaves; attached peduncules of tomato and pepper fruits; green lemon, grapefruit and orange fruits; slices of potato and carrot kept in Petri-dishes with water.

Inoculations were made as follows:

a) Specimens were pricked with a needle through drops of sterile distilled water suspension of a 24 or 48 hours old nutrient + 1% glycerin agar slant culture.

b) Specimens were sprayed with the suspension.

c) Leaves were first water-soaked by a fine syringe charged with distilled water and held about an inch from the under side of the leaf, then smeared with the suspension.

Detached specimens and the plants were covered with bell jars over water, or without water. Attached fruits were enclosed in cellophane bags.

Control specimens were treated with distilled water instead of the suspension.

Unattached specimens were kept at temperatures of 12—14, 18, 20, 23, 25, 30, 33 and 37°C; plants in pots at 23—25°C.

RESULTS

Effect of infection conditions

Both isolates responded similarly to infection conditions, and no significant differences were found among the various types of colony.

Drops of suspension of both isolates applied to the hosts without pricking gave negative results in all cases.

Positive results were obtained: a) by pricking the material through drops of the suspension, and b) by water-soaking the leaves and smearing them with the suspension of the organism.

In all instances where positive results were obtained with the original isolates, similar results were obtained with inoculations of their reisolations.

Progress of infection was more rapid at temperatures of 20—25°C than at 12—14, and 30°C. No infection occurred at 33—37°C.

Inoculated specimens kept in stoppered jars or bell jars over water showed more rapid and greater infection at a given temperature than those kept in bell jars without water.

Infection progressed more rapidly on detached than on attached material under similar conditions.

Infection progressed more rapidly and was more extensive on leaves inoculated by the water-soaking method, than on those pricked in 3—4 places through drops of suspension.

Pure cultures from infected parts of inoculated hosts were isolated on nutrient and potato glucose agar plates at 25°C. Isolations recovered were identical with the original. Histological sections showed the presence of bacteria in the parenchyma cells and vascular elements of infected parts.

Control specimens remained healthy throughout the experiment, and showed only very tiny spots at the point of puncture.

Description of the lesions

Positive results were obtained with both isolates on all hosts tested; both isolates produced similar lesions on each host as described below.

1. Onion leaves and tomato fruits. — Lesions similar to those observed on the original onion leaves appeared on the leaves inoculated both with the onion and tomato isolates. They appeared as water soaked transparent spots which spread far beyond the centre of infection (Figure 1). In many cases the spots coalesced and produced softening of the entire leaf.

The inoculated green tomato fruits showed soft brown spots which were far larger (5—8 mm in diameter) than those observed on the original fruits (Figure 2). On detached fruits in stoppered jars the spots coalesced and a soft rot of the entire fruits ensued about 8—10 days after inoculation at 23—25°C.

2. Pepper fruits. — Infected detached and attached fruits showed soft, brown, flat spots which attained a diameter of 4—5 cm on detached fruits kept in stoppered jars over water, 5 days after inoculation at 23—25°C (Figure 3). The spots coalesced and a soft rot of the entire fruits ensued 3—4 days later.

3. Tomato and pepper leaves. — Soft, flat, light brown spots appeared around the centre of inoculation on attached pricked leaves, attaining a diameter of 4—6 mm 4 days after inoculation. In a few cases infection spread along the vessels, covering the entire leaf, on plants covered with bell jars over water. On detached leaves in stoppered jars over water, the lesions appeared first as soft, flat, distinct, brown spots which then spread rapidly along and between the vessels, and covered the entire surface of the leaves 8 and 3—4 days after inoculation at 23—25°C on specimens treated by the pricking and watersoaking methods, respectively, causing watery softening of all the leaves.

4. Potato leaves. — Soft, wet, light brown spots appeared on the inoculated leaves the day following inoculation (Figure 4). The lesions spread along and between the vessels, and covered the entire leaves 3—4 and 10 days after inoculation at 23—25°C on specimens treated by the water-soaking and pricking methods respectively.

5. Bean leaves. — Soft, dark-brown spots appeared around the centre of infection 1—2 days following inoculation. The lesions spread along and between the vessels

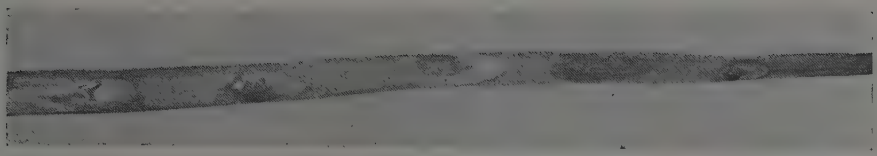

Figure 1

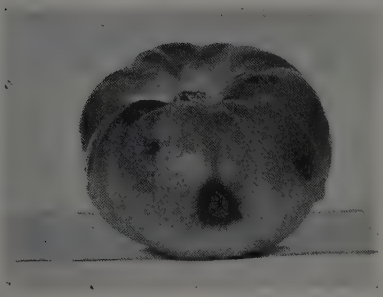

Figure 2

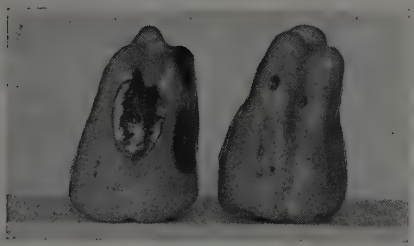

Figure 3

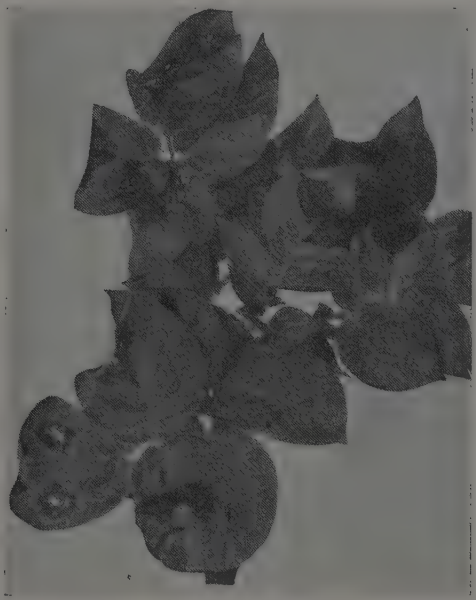

Figure 4

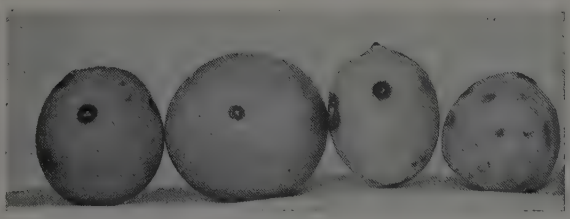

Figure 5

Figures 1 — 5. 1. Inoculated onion leaf showing water-soaked lesions around infection centres, 2 days following inoculation at 25 °C.—2. Inoculated green, detached, tomato fruit showing brown soft spot at 25 °C, 2 days after inoculation. — 3. Inoculated green, detached pepper fruit showing soft brown spots at 25 °C, and no signs of infection on the fruit kept at 32 °C, 5 days after inoculation. — 4. Green potato leaves inoculated by the pricking method, showing brown soft spots, 2 days after inoculation. — 5. Green orange, grapefruit and lemon fruits showing typical black pit lesions 3 days after inoculation; the control lemon at the end of the row shows punctures, signs only.

and covered most parts of the pricked leaves 10—12 days after inoculation, on plants covered with bell jars over water at 23—25°C.

6. Peduncules of tomato and pepper fruits. — Dark brown spot appeared around the inoculation centre on the peduncules. No infection, however, was observed to spread from the peduncule into the fruits.

7. Green lemon, grapefruit and orange fruits. — Typical black pit lesions appeared on the inoculated citrus fruits (Figure 6). Dark-brown, slightly sunken spots appeared around the inoculation centres, which attained a diameter of 4, 6 and 6—8 mm on grapefruit, lemon and orange fruits, respectively, at 23°C.

8. Slices of potato and carrot in Petri-dishes with water. — A cream yellow growth appeared on the slices, which were entirely softened 4—5 days after inoculation at 25°C.

DISCUSSION AND CONCLUSIONS

It appears that:

1) both isolates of the organism infect the plants through, or without, wounds; very likely through stomata in the latter case;

2) they attack the tissue of the plants at relatively low temperatures (12—14°C) and require a fairly high humid atmosphere to invade the tissues, and

3) they do not cause any infection at temperatures above 33°C.

Though the causal organism is closely related to *Pseudomonas syringae* Van Hall, and though both the causal organism and *Ps. syringae* caused black pit lesions on citrus fruits, infection experiments carried out several years ago with avocado and lemon isolates of the latter (Volcani 1950) indicate that there is one important difference. The avocado and lemon isolates did not cause soft rot in tomato fruits, nor did they produce softening of carrot and potato slices in the presence of water.

Since the tomato and onion isolates do produce such soft rot, the causal organism cannot be identified as this variety of *Ps. syringae*. Nor can it be identified with a new variety, *Bact.* (*Pseudomonas*) *syringae* var. *capsici* Orsini, reported by Orsini (1942) to cause soft-rot of pepper pods, since this variety is chiefly characterized by its non-pathogenicity to lemon, lilac and pear, while the tomato and onion isolates are pathogenic to lemon.

It is suggested, therefore, that the organism isolated from the tomato and onion lesions be identified as a different variety of *Ps. syringae* Van Hall, along with other strains of the same species, which are capable of inducing both soft-rot and black pit lesions on citrus fruits. A strain of *Ps. syringae* inducing yellowish-green soft-rot in potato slices was recorded by Rudd Jones and Dowson (1950). It was isolated from rotting potato tubers.

ACKNOWLEDGEMENT

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AUXIN AND INHIBITORS IN CANES OF *VITIS* *

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The effect of buds on rooting of grape cuttings (van der Lek 1925) as well as their response to synthetic growth substances (Evenari and Konis 1938, Pearse 1948) have been demonstrated. Furthermore, we have shown that leaching of cut canes in water for periods up to 96 hours influenced most favourably the percentage of rooting of different grape species and hybrids (Spiegel 1953). As the water extract of the grape stock *Vinifera* x *Berlandieri* 41 B, which strikes root with difficulty, had a somewhat deleterious influence on the easily rooting *V. Vinifera*, a study of the auxin and possibly the inhibitor content of the grape cane was indicated.

METHODS

The "free auxin" content was determined with the aid of the *Avena* test. The modified, "deseeded *Avena*" method (Skoog 1937) has been used under standard conditions (Avery, Creighton and Hock 1939, Went and Thimann 1937). As no gain whatsoever was found to result from the pulling out of the primary leaf in the deseeded *Avena* test, this practice was discontinued in further work. Victory seed (from Wageningen) was used, and results were expressed as microgram eqvs of IAA per kg of fresh weight (van Overbeek 1944). Short period extraction procedure did not deviate essentially from that described by van Overbeck (1938). Peroxide-free ether was employed. Buds and internodes of grape canes were analysed separately, 5 g fresh weight being used in each determination.

In some of these experiments auxin and inhibitor content have been assayed, parallel with similar determination in the *Avena* test, by means of the *Lepidium* method developed by Moewus (1948, 1950). These, however, gave less interpretable results than the *Avena* test. According to Went, additional factors present in extracts might influence growth response of roots. With certain dilutions of some extracts growth increments amounting to well over 16 percent above the control were obtained, this being the maximal growth increment elicited by the optimal (10^{-11} g/cm^3) concentration of IAA in this test.

CO_2 output has been measured by a modification of the Holdheide apparatus (Holdheide, Huber and Stocker 1936), essentially similar to that employed by Damast (1949). Results were expressed in mg CO_2 per kg fresh weight per hour. Five cuttings averaging 30 cm in length were used. Their combined weight amounted to about 100g. Measurements referred to were carried out at 25°C, with three replicates. Most of the investigations were carried out with buds and internodes of the following grape stocks: 1202, a *Vinifera* x *Rupestris* cross, cuttings of which strike root easily, and 41B, a *Vinifera* x *Berlandieri* cross, striking roots only with difficulty.

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RESULTS

Ether extracts obtained from internodes of the *V. Berlandieri* hybrid from October onwards gave rise to positive curvatures in the *Avena* test, even with the standard *Avena* method. Further work showed that water extracts from shoots of this variety strongly inhibited the growth of *Lepidium sativum* roots. Similar dilutions of *Vinifera* x *Rupestris* water extracts caused only slight inhibition. Inhibition was decidedly more pronounced with extracts of shoots derived from heavy soil than with those from light soil. Osmotic factors, though, might have been involved in the latter case.

Table I shows the inhibiting action of some extracts, as determined by Moewus' method (1948).

TABLE I

Inhibitory action of substances in water, ether, and alcohol extracts from grape canes (expressed as coumarin eqv. per 1 cm^3 of extract)

Grape species or hybrid	*Percentage of rooting*	*Water extract*	*Alcohol extract*		*Ether extract*		
Date of sampling		5/10	20/1	24/2	2/12	10/1	1/4
Vinifera x *Berlandieri* 41B	37	50	60	200	100	1200	300
Berlandieri x *Rupestris* 110	46	30	60	—	—	500	—
Berlandieri x *Rupestris* 99	58	—	—	—	—	500	—
Solonis x *Rupestris* 216-3	64	120	10	—	—	—	—
Vinifera x *Rupestris* 1202	69	20	20	20	—	—	—
V. Rupestris	72	0	—	—	—	—	—
V. Vinifera	75	0	—	0	—	—	0

The first column of Table I gives the average rooting percentage as determined for 1000 cuttings of each variety. It can be readily seen that the easily rooting species and hybrids showed a much lower inhibitor content in this test.

Cryoscopic determinations of the osmotic value of the extract have shown that this did not prove the dominant factor in the growth inhibition observed, while pH values of the cell sap bore no relation to the amount of inhibition assayed. Therefore it was thought possible that an excess of auxin might be involved. However, the addition of suboptimal concentrations of IAA shifted the curvature further to the negative side, as shown in Table II. This definitely rules out the possibility of the inhibiting action of the extract being due to excessive concentrations of IAA.

TABLE II

The effect of the addition of β-indoleacetic acid to ether extracts of grape internodes on curvature produced in deseeded Avena coleoptiles (+ indicates positive,—negative, curvatures)

	Curvature degrees
Indoleacetic acid, 5 γ/l	—5.4
Vinifera x *Berlandieri* 41B internodes	+2.1
Vinifera x *Berlandieri* 41B internodes + IAA 5 γ/l	—3.2
Vinifera x *Rupestris* 1202 internodes	—3.3
Vinifera x *Rupestris* 1202 internodes + IAA 5 γ/l	—7.1

Seasonal trend of the auxin inhibitor system and the CO_2 output of grape internodes during dormancy

A definite correlation has been established between the curvature obtained in the *Avena* test with grape cane extracts and the rate of CO_2 output of the cane segments.

Respiration (as measured by CO_2 output) was found to be consistently higher throughout the season in the *Vinifera* x *Rupestris* cross than in the *Vinifera* x *Berlandieri* cross, as can be readily observed in Figure 1. A steep rise in respiration rate becomes evident in both stocks somewhat prior to the stage when negative curvatures reach an appreciable level. Throughout the whole period of study, auxin activity was found to be higher in the more easily rooting *Vinifera* x *Rupestris* cross. Curvatures produced by ether extracts of *Vinifera* x *Berlandieri* proved to be positive during most winter months, indicating a preponderance of inhibitors over auxin. Only after April 1st negative curvatures begin to appear in the *Berlandieri* hybrid, indicating higher auxin than inhibitory activity.

Further work has demonstrated that, if these ether extracts are repeatedly shaken with weak aqueous solutions of $NaHCO_3$ or Na_2CO_3, the auxin goes over into the alkaline solution and can be reextracted from it with ether, after due acidification.The residual ether solution accounts then for all inhibitory activity. In the unfractionated extract, the inhibitor very often masks auxin activity, and positive curvatures do result, esp. with *Vin.* x *Ber.* 41B. Contrary to this, the acid extracts separated by the above mentioned procedure and tested by the *Avena* method have an appreciable auxin content even in January, in both hybrids, as can be seen fron Figure 2.

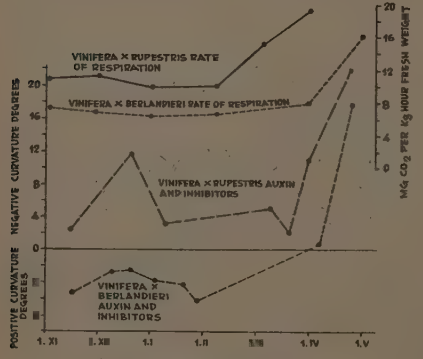

Figure 1

Rate of respiration (expressed as mg CO_2 per kg fresh weight/hour) and auxin-inhibitor values (expressed as curvature degrees in the Avena test) of grape cane internodes.

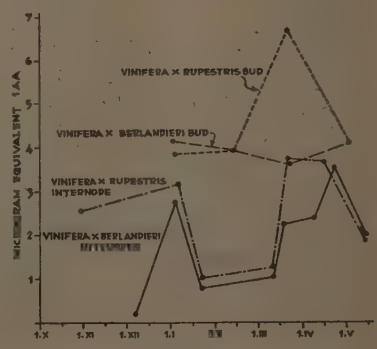

Figure 2

Free acid auxin content (expressed as microgram eqv. IAA per kg fresh weight) in buds and internodes of two grape hybrids.

The auxin values for the internode were quite similar in both stocks, but the rise in spring occurred earlier in the *Vinifera* x *Rupestris* cross, which showed also much earlier leafing: swelling of buds on March 20, as against April 17 in the case of *Vinifera* x *Berlandieri*. The sharp rise in bud auxin values was noted only with the *Vin.* x *Rup.* cross. The amount of auxin was usually higher in buds than in comparable internode sections.

While well defined auxin activity was apparent in the acid fractions, the neutral fraction gave rise to noticeable positive curvatures, as shown in Figure 3. Inhibitor

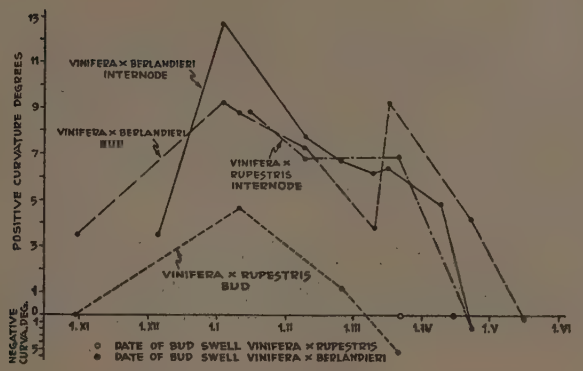

Figure 3

Inhibitory activity in neutral fraction of ether extracts from grape cane buds and internodes (expressed as positive curvature degrees in the *Avena* coleoptile).

activity is well evident also in the *Vinifera* x *Rupestris* cross, though activity of the inhibitor is found to disappear from the buds somewhat prior to bud swelling, and at a later date from the internodes. The buds have higher auxin values towards spring than the internodes. Following the disappearance of the inhibitor from the neutral fraction, slight negative curvatures are obtained in the *Avena* test, indicating presence of some neutral auxin.

It is striking that inhibitor activity ceases at a much earlier date in the *Vinifera* x *Rupestris* cross than in the *Vinifera* x *Berlandieri* cross. The *Berlandieri* hybrid showed a much later inception of activity in spring, as indicated by late bud swelling, very late rooting, and low respiratory activity.

In addition to investigations of the auxin and inhibitor content in natural termination of dormancy, determinations have been made with canes undergoing cold treatment. It was found that two and a half months of chilling at 2 °C seemed to have caused nearly complete disappearance of inhibitor activity in the neutral fraction of grape cane ether extracts.

Identification of native auxin in the grape

The acid nature of auxin in *Vitis* became evident. Methods which have been employed to ascertain identity will be summarized briefly.

1) *Acid-alkali stability*. While the unfractionated ether extract gave an 8.7° negative curvature in the deseeded *Avena*, the treatment with HCl destroyed most of the auxin activity, and brought about a 1.2° positive curvature. Alkali treatment of a similar concentration (0.1 N) even caused an increase (up to 14.0°) in the negative curvature. This might indicate release of protein-bound auxin (Wildmann and Bonner 1947) or presence of an alkali-sensitive inhibitor. Alkali stability and acid sensitivity are known to be (Bonner 1950) properties of indoleacetic acid.

2) *Enzymatic destruction* Treatment of grape cane ether extracts with indole acetic acid oxidase obtained from etiolated pea stems almost entirely destroyed auxin activity in

these extracts. The 1:10 active dilution of the pea extract (Moewus 1950) was used. After treatment with this dilution *Lepidium* roots grew at the same rate in diluted grape cane extracts as in water controls. The untreated grape extract of the same dilution brought about a 44 percent inhibition in the *Lepidium* test. Higher dilutions of grape extracts stimulated rate of growth of *Lepidium* roots but they failed to do so after treatment with pea extract. As the pea oxidase is considered (Tang and Bonner 1947, Wagenknecht and Burris 1950) highly specific for IAA, further evidence for its presence was called for.

3. *The Salkovski test.* A large bud sample (from the *Vinifera* x *Rupestris* cross) weighing 200 g was extracted with peroxide-free ether and repeatedly purified according to the method given by Wildmann and Bonner (1948). This was carried out on March 20, just prior to bud swell, the date coinciding with auxin activity as indicated by biological tests. With the aid of the Salkovski reagent (Gordon and Weber 1951,

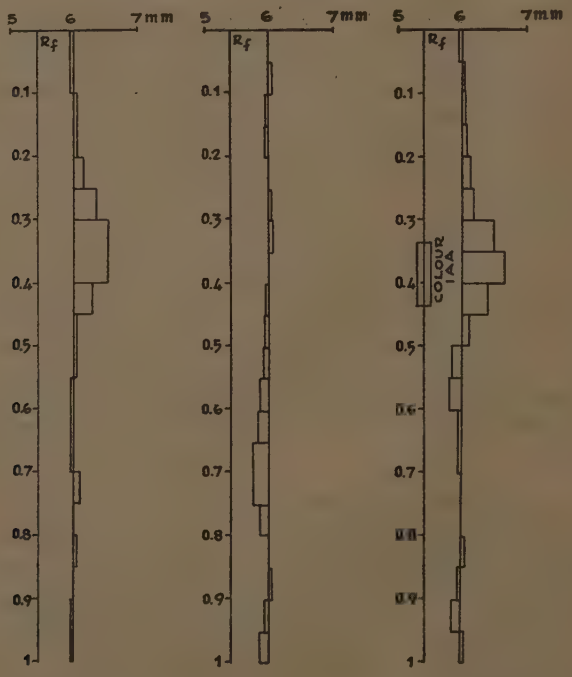

Figure 4

Growth increment of etiolated oat coleoptiles on a paper chromatogram of fractionated ether extracts of *Vinifera* x *Berlandieri* 41B canes. On the left: acid fraction; on the right: neutral fraction. Butanol-ammonia-water solvent. Values represening average of 20 coleoptiles, expressed as 0.01 mm.

Figure 5

Growth increment of etiolated oat coleoptiles on chromatogram from unfractionated ether extract of *Vin.* x *Berl.* canes. Isopropyl alcohol-ammonia-water solvent. Note location of red spot with IAA (Salkovski reaction).

1951, Wildmann and Bonner 1948) a somewhat clouded pink solution was obtained. Spectrophotometric measurements indicated a concentration of 4.5 μg IAA per kg fresh weight (a similar order of magnitude as that indicated by the coleoptile method).

4. *Chromatographic separation.* Further proof of the presence of IAA was obtained by chromatographic means. The solvents used were similar to those employed by Luckwill (1952) and Bennet-Clark et al. (1952). The growth increment of 5 mm long *Avena* coleoptile segments on sections of paper chromatogram was measured.

Figure 4 shows an increase in growth and sometimes decrease in various segments, as compared with growth in water. The vertical axis represents the growth increment of the water control and deviations from this line indicate increase and decrease of growth rate in extracts. While the acid fraction shows a definite peak at R_f 0.3—0.4, the neutral fraction shows only a negligible increase at the same R_f, with 2 inhibition zones clearly marked: a larger one, at R_f 0.65—0.75, and a smaller one at R_f 0.95—1.00.

Figure 5 shows the chromatogram of the unfractionated extract and also the R_f at which a red spot was obtained by action of the Salkovski reagent on synthetic IAA. Taking into account the coincidence of both locations, auxin activity in the acid fraction of ether extracts of the grape was shown to be due to IAA. While the chromatographic separation thus clearly showed the identity of the growth promoting substance, R_f of probably two inhibitors has also been indicated.

DISCUSSION

The identification of the native auxin in grape canes as indoleacetic acid is in agreement with recent views concerning the nature of auxin (Bonner 1950). It is noteworthy that the highest content of IAA during winter months was found in the buds of *Vinifera* x *Rupestris* cross just before bud break (about 6.7 IAA mg eqvs. per kg fresh weight). There was also an indication of the presence in the neutral fraction of a neutral auxin, though in small quantities (Figure 3). The activity which might have been due to indoleacetaldehyde (Larsen 1949) or indolylacetonitrile (Jones et al. 1952), became apparent only after the complete disappearance of an ether soluble inhibitor in that fraction. Inhibitor activity was quite pronounced even in the unfractionated extract, often masking auxin activity and measurements by biological methods. A sharp rise in rate of respiration was noted somewhat prior to the disappearance of the inhibitor from the neutral fraction of ether extracts. While we could thus connect the termination of dormancy with the presence of a neutral inhibitor, Hemberg (1949, 1952) found that the content of an acid inhibitor declines sharply before sprouting of potato tubers.

In accordance with his findings (Hemberg 1952), cold treatment also brought about a strong diminuition in inhibitor content. Auxin-inhibitor relationship during dormancy has been reviewed by Hemberg (1947) and Samish (1954).

While we found quite noticeable auxin activity in ether extracts of the grape during dormancy, no appreciable auxin activity was found during winter with the agar diffusion method, thus confirming Zimmermann's results (1936). However, the relation between ether extractable auxin and growth phenomena, as postulated by Went (1942), has been confirmed. Respiration rate was also shown to parallel the apparent auxin

level. The relation between root differentiation and auxin level established by Fischnich (1939) and Stoughton (1938) has also been shown to exist in the grape. Also Warmke and Warmke (1950) and Skoog and Tsui (1951) have shown lately that, while low auxin concentrations in the tissues favour bud formation, high auxin levels are conducive to root differentiation. With us, the easily rooting *Vinifera* x *Rupestris* 1202 gave evidence throughout winter months of higher rooting percentages and significantly higher curvatures in the *Avena* test than the less readily rooting *Vinifera* x *Berlandieri* 41B. For most of the winter period the latter had a high inhibitor content masking auxin effects in unfractionated extracts. A great many of the auxin measurements reported in literature could have been similarly influenced by high inhibitor content. In our work inhibitors were shown to be important factors in the difficult rooting *Berlandieri* hybrids, as they were found both in water and ether extracts and also alcohol extracts of the canes thereof.

Chromatographic evidence suggests the presence of two inhibitors. This is the first time that a definite relationship between inhibitor content and rooting response has been established. Further investigation on this subject seems indicated, since our previous work has shown that the water leachings of the poorly rooting *V. Berlandieri* cross adversely affect the rooting of other grape species.

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SEASONAL FLUCTUATIONS IN FERTILITY AND OTHER CHARACTERISTICS OF BULL SEMEN USED FOR ARTIFICIAL INSEMINATION IN ISRAEL * [1]

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Defective fertility had long been a source of serious concern to dairy cattle breeders in Israel. Since the trouble has been fully realized in recent years as a result of the widespread use of artificial insemination by large breeders' cooperatives, attention has been focused on the problem of semen quality.

To render possible correlation of the results obtained with the prevailing weather conditions, a short description of the Israel climate is given. Israel belongs to the region of Mediterranean or Dry-Summer Subtropical Climate, which is defined by Trewartha (1943) as follows:

"In its simplest form this climate is characterized by three principal features: (a) a concentration of the modest amount of precipitation in the winter season, summers being nearly or completely dry; (b) warm to hot summers and unusally mild winters; and (c) a high percentage of the possible sunshine for the year and especially in summer."

August is normally the hottest month of the year, but the average July temperature is often as high. The summer heat continues in September, and the average daily temperature in October is still higher than in May.

Anderson (1945) maintains that warm conditions are conducive to the production of good quality semen in Kenya. Mercier and Salisbury (1947) are of the opinion that in Canada increasing daylight stimulates fertility and the effect may continue well beyond the longest day. It appears, however, from their data that the higher summer temperatures may also constitute a favourable factor. In Nebraska (Morgan and Davis 1938; Schulze, Davis, Blum and Oloufa 1948), Indiana (Erb, Andrews and Hilton 1942), Missouri (Weeth and Herman 1949) and Maryland (Hilder, Fohrman and Graves 1944; Phillips, Knapp, Hemmstra and Eaton 1943), the peak of fertility occurs in spring and the minimum between June and September. The effect of high temperature can manifest itself immediately (Hilder et al. 1944, Phillips et al. 1943, Schultze et al. 1948), or there may occasionally be a lag of one month [5,10]. In most cases the depression extends long up to two months after the hottest month.

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The present study deals with the monthly and seasonal variations in fertility of semen, as expressed in the rate of conception obtained by artificial insemination, and in some other qualities of the semen.

Our observations were based on semen obtained from Dutch and Holstein-Friesian sires, while the cows were pure Dutch and Damascus × Dutch grades of the third to fifth generation. The technique of collection, dilution and preservation, followed the accepted methods. Sperm concentration, methylene blue reduction time, and initial motility of the spermatozoa, were determined for all ejaculates. A slightly diluted sample was preserved at 4 °C for longevity tests. Conception rates were computed only from inseminations of cows which did not show any abnormalities in their genital organs or disturbances in oestral cycle and which conceived after not more than four inseminations.

SEASONAL VARIATIONS IN FERTILITY

Fertility, as expressed by the percentage of conception, is lowest in September and October, when it falls 8—14 per cent below the annual average. The decline begins in August or even in July; in extreme cases as early as June (Figure 1). The highest rate of conception occurs from March to May. During that period it exceeds the annual average by 8—10 per cent. In some bulls the high fertility level is confined to these months only; in others, it is maintained until June or July. In some cases the rate of conception in January and February is as high as in the spring.

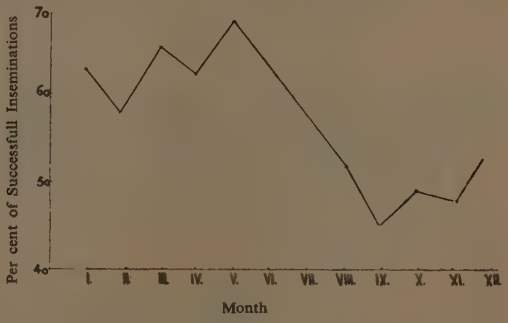

Figure 1
Monthly variations in percentage of conception

VARIATIONS IN FERTILITY OF STORED SEMEN

For the determination of variations in fertility of stored semen, conception percentages were calculated separately from inseminations carried out on the same day, as well as one day and two days after ejaculation. The effectiveness of inseminations with stored semen equalled the results obtained with fresh ejaculates during the major part of the year. A lowering of fertility in storage was recorded from August till October. This autumn depression of fertility of stored semen corresponds to the decreased longevity of stored spermatozoa during that season (Figure 2).

THE INFLUENCE OF SEASON ON LONGEVITY OF SPERMATOZOA

The following criteria were applied for expressing longevity: 1) the period after ejaculation during which the semen maintained a motility percentage not lower than 40, and 2) the time after ejaculation when motility ceased altogether. The difference

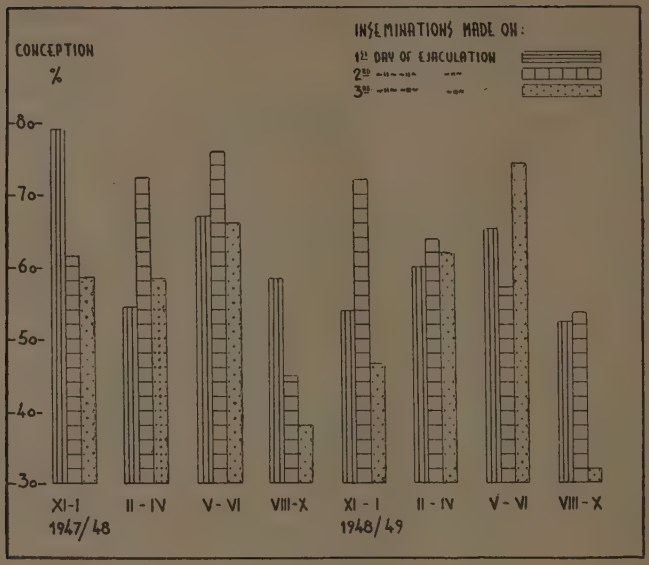

Figure 2
Seasonal variations in fertility of fresh and stored semen

between the shortest and the longest term of longevity was in the region of 60 and 30 per cent for the first and second criterion, respectively.

The peak of longevity is reached in May and June. In July the lifespan of the spermatozoa begins to decline and reaches the lowest level in September and October. It resumes a gradual upward trend in winter and spring (Figure 3).

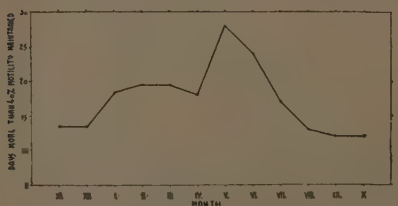

Figure 3
Monthly variations in longevity of spermatozoa

MONTHLY VARIATIONS IN VOLUME OF SEMEN PER EJACULATE AND CONCENTRATION OF SPERMATOZOA

Although the volume of ejaculate of some bulls seemed to decline between August and October, no significant differences in semen volume were found as between different months.

The highest concentration of spermatozoa was found in June (115—130% of the annual average) and in some bulls it remained fairly constant at that level well into July and August. The minimum concentration was recorded in September and Octo-

ber (70—90% of the annual average). In winter the concentration was low but it increased gradually as the season progressed. Some relation was indicated between the average annual concentration levels and the age of the sires. However, the age at which the highest concentration of spermatozoa occurred varied with individuals.

MONTHLY FLUCTUATIONS IN METHYLENE BLUE REDUCTION TIME

A method of computation of *relative reduction time*, which takes into account in the interpretation of results the concentration of spermatozoa has been evolved[7]. It seems that the values thus obtained provide a fairly accurate measure of semen fertility.

The relative reduction time is longest from August to October. Following a gradual decrease in November and December, the lowest values are obtained from January to June.

DISCUSSION

The seasonal fluctuations in semen fertility in Israel resemble roughly those recorded in some parts of the United States, but the period of low fertility continues in this country until October. The high fertility phase ends in most cases in June, but occasionally extends till July.

Both photoperiod and temperature probably play an important role in determining the fertility level. The increasing length of day in the spring may be assumed to favour fertility, though this effect is counteracted by the advent of high temperature in June. The effect of high temperature is mostly immediate but occasionally there is a lag of one month.

The depression of fertility continues until October — two months after the inversion point of the temperature curve —, although the average daily temperature in that month is slightly lower than in June, when fertility is considerably higher. The fact that the seasonal decline of fertility begins earlier and is more pronounced in some bulls may be the result of differences in adaptability to the climatic conditions of Israel.

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SOME OBSERVATIONS ON THE OXIDATIVE DESTRUCTION OF LYCOPENE DURING THE MANUFACTURE OF TOMATO PUREE

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In the manufacture of tomato puree it has been found difficult to produce a bright red colour from tomato varieties grown at present in Israel. This investigation was carried out during the 1953 tomato season in order to establish the factors responsible for deterioration of colour in the finished product.

The use of certain only slightly coloured varieties of tomatoes for the manufacture of tomato puree is indeed not advisable, but apart from special instances a satisfactory colour can be obtained by application of the following observations.

The carotenoid lycopene ($C_{40}H_{56}$), present in ripe tomatoes in amounts of up to 0.40 g per kg, is responsible for the red colour of the fruit (Karrer et al. 1928, 1929, 1930, 1931; Karrer 1939, Kuhn 1932). This hydrocarbon is sensitive to both oxidation and isomerization, two procedures that decrease colour intensity.

METHODS

The method of W. B. Davis (1949) was adopted, with some changes. According to this method the pigments are extracted with acetone, and the extract is analysed spectrophotometrically.

Chloroform was substituted for acetone because of the higher solubility of lycopene in chloroform. Enriched lycopene solutions where only traces of yellow pigments remained were obtained through repeated treatments of the pulp with 96% alcohol before extraction by chloroform. Their purity was checked from time to time chromatographically. A Coleman Junior spectrophotometer at a wave length of 500 mμ was used for measuring per cent transmittance of lycopene solutions. The results are expressed in p.p.m. lycopene. The standard curve was prepared with solutions of known concentration of lycopene in chloroform. This lycopene was prepared by washing the pulp 3 times with 96% ethanol, extracting by chloroform, and recrystallizing 3 times from chloroform. Chromatographic tests revealed no traces of other pigments in lycopene prepared in this way.

INFLUENCE OF HEAT TREATMENT ON COLOUR OF TOMATO JUICE

Fresh tomato juice was subjected to different heat treatments *in vitro:* the influence of heat on colour was determined by measuring the losses of lycopene. These tests were carried out only as a means of orientation, but we tried to duplicate as closely as possible conditions prevailing in the processing line (Table I).

Allowance was made for an increase in the concentration of the juice, a consequence of evaporation. This increase was indicated by an increase in the refractive index. Length of cooling time had a decided influence on the destruction of lycopene.

TABLE I

Heat treatments of fresh tomato juice

	Steam bath			*Boiling on open flame*		
	P.p.m. lycopene	*Absolute loss*	*Relative loss %*	*P.p.m. lycopene*	*Absolute loss*	*Relative loss %*
Fresh juice	43.0					
	32.0					
	36.8					
	43.4					
1 minute quick cooling				42.8	0.2	0.47
				31.5	0.5	1.56
				36.8	—	—
				—	—	—
1 minute slow cooling				40.0	3.0	7.0
				30.0	2.0	9.4
				34.0	2.8	7.63
				—	—	—
5 minutes quick cooling	41.6	1.4	3.26	—	—	—
	—	—	—	31.0	1.0	3.12
	—	—	—	36.0	0.8	2.17
	41.7	1.7	3.9	—	—	—
5 minutes slow cooling	39.6	3.4	7.9	39.1	3.9	9.1
	—	—	—	28.4	3.6	11.2
	—	—	—	31.8	4.2	13.6
	37.6	5.8	13.4	—	—	—
10 minutes quick cooling	40.6	2.4	5.59	—	—	—
	—	—	—	29.1	2.9	9.07
	—	—	—	34.2	2.6	7.07
	40.0	3.4	7.65	—	—	—
10 minutes slow cooling	36.3	6.7	15.6	34.3	8.7	20.2
	—	—	—	26.0	6.0	18.7
	—	—	—	28.1	8.7	23.8
	34.0	9.4	21.6	—	—	—
30 minutes quick cooling	37.5	5.5	12.8	—	—	—
	—	—	—	27.9	4.1	12.8
	—	—	—	33.0	3.8	10.3
	38.9	4.5	10.4	—	—	—
30 minutes slow cooling	30.0	13.0	30.2	30.7	12.3	28.6
	—	—	—	23.0	9.0	28.1
	—	—	—	24.3	12.5	34.0
	30.1	13.3	30.7	—	—	—

Results obtained by steam-bath heating differ only slightly from those obtained on the open flame, in spite of the considerable difference in temperature (80—85°C, against 100°C). These results suggest that the length of heating period, rather than the height of temperature, is the factor controlling losses of lycopene.

Slow cooling was obtained by plunging containers filled with hot juice into 10 to 15 times their volume of water which had been heated to the juice temperature, and by

letting the whole system cool down to room temperature. This slow cooling thus duplicated a method often used in industrial practice, when tanks of hot juice are allowed to cool down for a while.

OBSERVATIONS CARRIED OUT IN THE FACTORY

The second part of our work was concerned with the losses of lycopene due to various operations in the manufacture of tomato puree.

The average content of lycopene was measured in fresh juice, in hot juice entering the balance tanks before concentration, in samples drawn from the balance tank after slow cooling, and in the finished batch of puree. The losses of lycopene are summarized in Table II.

TABLE II

Fresh juice p.p.m. lycopene	*Juice from storage tank*			*Puree diluted to single strength juice*					*Juice from storage tank slowly cooled*		
	P.p.m. lycopene	*Abs. loss*	*Rel. loss*	*P.p.m. lycopene*	*Loss during concentration*		*Total loss*		*P.p.m. lycopene*	*Total loss*	
			%		*Abs.*	*Rel. %*	*Abs.*	*Rel. %*		*Abs.*	*Rel. %*
28.0	25.8	2.2	7.85	24.4	1.4	5.42	3.6	12.8	20.3	7.7	27.5
29.2	28.0	1.2	4.11	26.0	2.0	7.15	3.2	10.9	21.0	7.0	24.0
20.0	19.6	0.4	2.0	19.1	0.5	2.55	0.9	4.5	16.0	7.6	38.0
39.4	39.0	0.4	1.02	38.2	0.8	2.05	1.2	3.04	31.6	8.4	21.3
43.4	42.6	0.8	1.84	42.0	0.6	1.40	1.4	3.22	34.4	9.0	20.3

Results show that serious losses of lycopene occur when holding time is too long. Here conditions for the destruction of lycopene are ideal: the high temperature and large amount of air dissolved in the juice during the breaking and straining operations quickly destroy sizeable amounts of lycopene. During evaporation smaller losses are noticed because the deaeration which occurs as soon as the juice enters the evaporating system hinders oxidative destruction of lycopene.

The higher viscosity of the puree, as compared with that of the juice, seems to slow down a further destruction of lycopene. This point is confirmed by analysis carried out on samples of the same puree, which were heat-treated for different periods of time without showing detectable losses of lycopene.

We are not inclined to think that the differences of pH between juice and puree are involved in the destruction of lycopene, because these differences are too small, as a result of the strong buffer effect shown by the system.

CONCLUSIONS

Of course more work has to be done on the present subject in order to understand thoroughly the mechanism of oxidative destruction of lycopene during the processing of tomato puree.

Nevertheless it may be stated that oxidative destruction is checked by deaeration and "high-short" heat treatment of the raw juice and by cutting down as far as possible the time between heat treatment of the juice and its feeding into the evaporating system.

Under no conditions should hot juice be held at a high temperature before evaporation longer than required for the inactivation of enzymes.

By an application of these preliminary results to industrial processing we have been able to obtain a puree of a satisfactory colour.

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JUNCUS MARITIMUS, A RAW MATERIAL FOR CELLULOSE

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When Palestine Potash Limited started producing chlorine needed for the Dead Sea bromine plant, an outlet was sought for irregular surpluses of the chlorine or alkali produced in the electrolytic process. Since these are used in large quantities in the cellulose industry (Pomilio and other digesting processes), Z. H. Littman and one of us, suggested use of some local grass species for cellulose production. The late G. Boehm (Pal. Pot. Ltd.) proposed *Juncus maritimus* and, in June 1947, tests were undertaken in the firm's laboratories.

Juncus maritimus is a desert grass plant which grows in considerable quantities in different parts of the Negev. It has been found to grow on soil where ground water rich in salt occurs; e.g., there are large areas of it by the springs of Ein Arus near Sdom, which contain a considerable percentage of salt (every litre contains 1,450 mg NaCl, 100 mg KCl, 900 mg $MgSO_4$, 400 mg $MgCl_2$ — besides calcium bicarbonate corresponding to 150 mg $CaCO_3$).

Already in earlier times the *Juncus* species seems to have been used as a source of fibres for different purposes. G. R. Boehmer (1794) wrote that in former times *Juncus maritimus* was used for making wicks for lamps. G. Watts (1890) wrote: "In India, none of the species appear to be utilised, but in Europe the common rush, *Juncus effusus* L., is employed for making mats, baskets, and chair bottoms, and its pith is used for the wicks of rush lights. In Spain it is said to be used also in the preparation of a textile fibre. Two other species are utilised for paper making in Australia, and in Italy one affords a cordage fibre. It is extremely probable that certain of our Indian species might be similarly utilised..."

It seemed to us worthwhile to look into the problem of using *Juncus maritimus* for cellulose production, since it grows in long blades uninterrupted by knots. This is in contrast to other grass species which are utilised for cellulose production, where the knots are always a disturbing factor in the production process.

In grass samples from the Sdom area we found an average cellulose content of 55.7% in the moisture-free grass (according to the method of Norman-Jenkins (1943). This result was, of course, taken as a preliminary one, because the cellulose content depends on the age of the grass, on the season of harvesting, etc. In any case, the result justified investigating the digestion of the grass. We used the soda process which is common for Esparto. Several tests were carried out to elucidate the most suitable conditions for digestion. The grass was cooked while being constantly stirred at 160°C in an autoclave with varying additions of caustic soda. After digestion, the pulp was filtered and

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washed. It was then bleached in two or more stages, using in the first stage chlorine water, followed by extraction of the pulp with 2% caustic alkali (on the pulp) at 40°C. After being washed, the pulp was bleached with hypochlorite (bleaching powder solution) at 40°C. Then, an after-treatment with SO_2 was carried out in order to remove all traces of chlorine. The experiments were made with 130 g, corresponding to 120 g moisture-free grass. The grass had been cut into pieces of about 5 cm. Before cooking, the grass batches were soaked with a corresponding quantity of alkali solution for eighteen hours. The ratio of moisture-free grass to the volume of caustic soda solution was always 1:4.5 — this ratio having been found to be most suitable in our working conditions.

The most important results of a large number of experiments are enumerated in the following table; the experiments lasted four hours each at a maximum temperature of 160°C.

TABLE I

	Concentration of caustic soda solution	*Parts of caustic soda*	*Yield of unbleached dry pulp*	*Yield of bleached dry pulp*	*Cellulose content in unbleached dry pulp*
	(*g/l*)	(*per 100 g moisture-free grass*)			%
1.	47.7	21.7	37.0	34.6	92.1
2.	47.7	21.7	37.3	34.8	92.2
3.	42.9	19.5	41.2	not determined	90.4
4.	42.9	19.5	42.1	39.4	90.5
5.	38.2	17.3	41.8	39.4	90.0
6.	33.4	15.2	45.8	38.9	83.7
7.	33.4	15.2	44.8	37.1	84.2

It will be seen that the most favourable results were obtained in digestions 3, 4, and 5; namely, in those cases where 17—19.5% caustic alkali was used. When using more alkali (digestions 1 and 2), the yield of unbleached pulp dropped. When using milder conditions (digestions 6 and 7), the yield of unbleached pulp rose; but the cellulose content of this product was lower and the chlorine consumption in the bleaching operation rose enormously.

The experiments showed that *Juncus* fibre can, without difficulty, be processed by the caustic soda cooking process, and there are good prospects that *Juncus* fibre could be in this respect competitive with Esparto.

At the time, it was impossible to test the pulp samples in this country. Therefore we submitted them — bleached and unbleached — together with original grass to the laboratories of Cross & Bevan, the British experts for cellulose products. Comparing the results obtained with the commercially prepared samples of bleached pulp from straw and Esparto, they summarized their findings as follows:

> "...it will be seen that the *Juncus* pulp is intermediate between the two sets of values, except for tear, in which the *Juncus* is superior to both... An economic yield of pulp could be obtained by normal alkali cooking and bleaching. The difficulties encountered in cooking will be those inherent in grass or straw processing, namely (a) low digestion capacities due to the bulk of the raw material and (b) possible difficulties in alkali recovery. The pulps so produced would be intermediate in properties between the pulps of Esparto and straw and as such could be used industrially in the ranges of papers in which these pulps find uses.

It is to be emphasized that the samples of pulp which we examined do not necessarily represent the optimum quality that could be produced. This point could be settled only by a complete range of digestions examining the effect of the different variables involved..."

Additional extensive laboratory experiments and large scale trials, taken up by Mr. Lewin of the Forest and Fibres Research Laboratory (formerly of the Research Council of Israel and at present of the Ministry of Agriculture), seemed to confirm essentially our preliminary findings. Using the soda process, a good quality pulp was obtained from *Juncus*. Various types of paper made from *Juncus* fibre proved to be generally of the same quality as those made from Esparto pulp (*Paper Trade Review*, 1953).

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THE SEIFS ON THE ISRAEL—SINAI BORDER AND THE CORRELATION OF THEIR ALIGNMENT

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This report is dedicated to the memory of the late Professor A. Reifenberg who brought me to geographic research and taught me the scientific evaluation of aerial photographs.

This report is mainly a study of vertical and oblique aerial photographs of various dates, and is concerned with the orientations and properties of longitudinal sand dunes and presents some new suggestions regarding the mechanism and alignment of seifs.

Although the longitudinal dunes in the Negev and Northern Sinai are very similar to the sand ridges of Australian deserts (Madigan 1936), and are not like the "seifs" of North Africa (Aufrere 1931, Capot-Rey 1945) or of Southern Arabia (Bagnold 1951) which are of dissimilar size and spacing, the term "seif" has been generally used for long parallel sand dunes and it will also be used here.

Out of a larger area situated on the Israel-Sinai border and covered with seifs, a smaller area of about 200 square kilometres was selected for detailed study.

The seifs of this area are of varying length — several up to eight kilometres long, between 50 to 100 metres broad and about 10 metres high, generally with a fairly flat top. They run in fairly straight parallel lines spaced about 150 metres apart.

The rump and thus the flanks of the seifs appear to be made of semi-consolidated material. Cornish (1908) already mentioned that dew effects may produce even in desert areas "enough moisture to consolidate the lower part of the dunes, but readily evaporating at the surface, thus allowing the top layers to be redistributed by the daily breeze." In our region, however, the retention of rain water, as explained by Brigadier Bagnold (1941), may be the main cause. Although loose sand is also found swept against the sides of the seifs, it is much more prominent in the form of little dunes on the top of the seifs, leaving the flanks mostly bared.

The azimuths of the seifs are found to be limited to a certain range of at most 30° divergence, the majority lying within an angle of 20°. Although there are some seifs running at random azimuth within the given angle of divergence, the great majority of seifs run in one of several specific azimuths which are sharply defined, with a tolerance of ± one degree, and which are not a mean of random directions. The seifs occur mostly in groups of a common specific azimuth of 270°, and also 259° and 249°, the decrease being from north to south.

From the pattern of the longitudinal dunes it is possible to recognize clearly certain individual seifs to be composed of fragments of seifs originally aligned in another direction (but also in one of the specific azimuths). Thus it appears that at times a group of parallel seifs alters direction by breaking up at some places and coalescing with

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other seifs at some distance. Such seifs have a somewhat zig-zag form instead of a fairly straight line.

On the top of seifs small transverse dunelets are very often found, and these may be likened to almost straightened-out barchans. These dunelets, too, have a characteristic orientation indicated by the azimuths of their long axes. On the top of the seif they form a wave-like pattern with a fairly defined "wavelength". In the area considered this repetition distance was about 50 metres. Often these dunelets slant from the bottom of the seif up the side, over the top and down on the other side. While preserving their azimuths and distances, the dunelets are found to shift in the direction of the longitudinal dunes, the displacement being of an order of several metres per annum (3.5 — 8 metres measured). In the course of seven years, a lateral shift has not been discernible.

These dunelets were also found on the comparatively firm ground of the flats in the dune area, where they accumulate in echelon, one obliquely displaced behind the other. In these accumulations they have the same azimuth and repetition distance as on the top of the seifs, while their height is estimated at about two metres and less. It is noteworthy that these accumulations have the same longitudinal azimuth as the seifs, and may possibly constitute seifs *in statu nascendi*.

The azimuths of these dunelets vary, in the area concerned, between 307° and 325°, the great majority lying between 312° and 318°. The lack of suitable further material prevented the establishment of specific dunelet azimuths, of which there was perhaps some evidence.

The nearest meteorological stations are at El 'Arish and Beersheba. Their somewhat erratic data regarding winds have recently been analysed by Rosnan (1953) with particular reference to seif alignments, and his results will be used here.

For the area studied only the wind directions of El 'Arish are considered relevant, on account of geographical location. Thus, the prevalent summer wind (July) at El 'Arish has an azimuth of 315° with a frequency of 32%, the figures for the prevalent winter wind (December) being 225° and 40%.

From the papers and discussions at the International Symposium on Desert Research, Jerusalem 1952, it seems permissible to infer that no particular correlation between wind directions and seif azimuths has so far been entirely uncontested. Brigadier Bagnold (1952) reported very good agreement with the resultant wind direction for a certain season, but not with the resultant for the whole year. On the other hand Rosnan found for the Northern Negev a very good agreement between seif azimuths and the resultant wind direction for the whole year, though this correlation might admittedly have been due to the particular distribution of wind with regard to its velocity and direction in the Northern Negev.

In view of this unsettled state I venture to submit tentatively a new method of correlating the azimuths of dunelets, seifs, and winds, based on the observations in the area studied.

As is seen from the data given, the dunelets are found to be aligned with their long axes (azimuth 312° — 318°) practically perpendicular to the direction of the prevalent winter winds (azimuth 225°) which are also the strongest here.

I shall try to deduce the alignment of the seifs from the vectorial displacement of the dunelets, as brought about by the winds throughout the year.

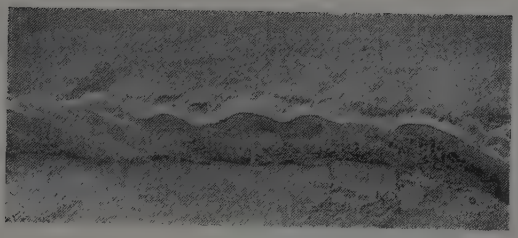

Figure 1
Seif with dunelets

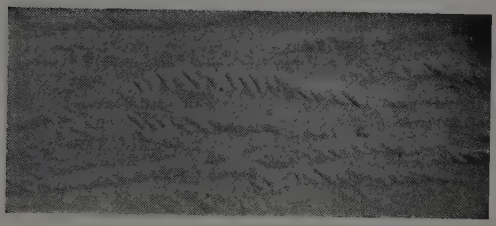

Figure 2
Dunelet accumulations

Figure 3
General appearance of seifs and dunelets
(Oblique view)

Using the notation of Brigadier Bagnold (1941) in his work on the theories of dune motion, the rate of advance of an isolated barchan on unit width c varies directly with the rate of sand movement Q and inversely with the height of the slipface h. In order to obtain a dependence on plane dunelet dimensions instead of dunelet height, which last is not obtainable from the aerial photographs used, I make the assumption that for dunelets the height h is proportional to their depth d, namely $h = \beta d$. Thus from $c = Q / \gamma\beta d$ it follows that the displacement $\underline{S}$ per unit time is proportional to Q/d.

As the dunelets found here do not have defined slip faces which would trap all oncoming sand, the application of this formula constitutes an approximation which appears, however, justified by the fact that for the straddling dunelets the flanks of the seif act as traps for the oncoming sand.

As Q varies with the third power of the wind strength, the displacement $\underline{S}$ in time t is given by the vectorial equation $\underline{S} = \alpha \sum (\underline{V}^3 \delta t/d)$, where $\underline{V}$ is the difference between the actual wind speed and a certain threshold value of wind velocity. A colloquial interpretation of this formula could be that the dunelet offers a resistance to the displacing wind, the resistance being proportional to the depth of the dunelet in the incident wind direction.

The yearly displacement can now be obtained by splitting the summation into two or three terms, according to winter, summer, and possibly also spring/autumn wind strengths, directions and durations.

For the particular area studied two approximations are now used, based on observations made:

1) It follows from Rosnan's (1953) paper that, owing to the peculiar wind regime in the Northern Negev, the sand displacement (uslng the above mentioned formulae) is roughly equal for a mean summer and winter month, the winter winds being much stronger but also much less frequent than the summer winds. No pertinent data being now available here for El 'Arish, I extrapolate this result, which was computed for Beersheba, to apply also to the region in its south.

Thus the $\underline{V}^3 \delta t$ factor becomes a constant per month, and the displacement S in a given azimuth is now proportional to the number of months and inversely proportional to the dunelet depth at wind incidence angle.

2) The form of dunelets here can be roughly taken as rectangular, the breadth (along the long axis of the dunelet) being about three times the depth.

Coming now to the computation proper, the winter wind being normal to the long axes of the dunelets, it will produce relatively the greatest displacement. Putting the dunelets' depth as unity, and taking the three months, December—February, as having specific winter winds, a corresponding displacement of three units in the 225° azimuth is obtained. Allowing for the two months of November and March, when the wind direction fluctuates between summer and winter azimuths, a mean azimuth of 270° and taking the displacing ability to be reduced by the above rule, i.e. by 1/1.28 = 0.78, a displacement of 2×0.78 units is obtained in the 270° azimuth.

The summer winds of seven months duration are almost exactly in the direction of the long axes of the dunelets and thus their displacing ability is reduced to one third unit, giving together $7 \times 1/3$ units in the 315° azimuth. The vector resultant of all these

displacements gives a displacement in the 275° azimuth, which agrees rather nicely with the main seif azimuth of 270° for the seifs in the regions south and south-east of El 'Arish respectively, i.e. for a region of about 1000 sq.kms.

South of the area considered lie nearby ridges which are extensions of the Jebel Helal massif. It seems plausible to assume that the summer winds are deflected to some extent by the massif and the ridges and thus add, for the area considered, a wind component which would otherwise not have appeared, and which will tend to reduce the azimuth of the displacement-resultant. The reduction would be most effective in the southern fringe and it would lessen towards north, and indeed this effect has already been quantitatively noted above.

It is also noteworthy that the seifs here appear to be comparatively steep on both the northern and the southern flank, with no outstanding preference. This seems due to the fact that both winter and summer winds possess components normal to the direction of the seifs, whose means compare as 0.55 to 0.8 of the respective wind strengths.

The presented method of deducing seif alignments from dunelet displacements reconciles perhaps the discordant findings regarding the effect of the wind resultant: on the one hand a certain season will give the main effect on alignment, while on the other, the method is actually a resultant for the whole year, though with different weights depending on the orientation of the dunelets.

The aim of this report is to draw attention to a possible correlation between the alignment of dunelets and seifs, and wind directions. The method presented appears to be adaptable also to resistances of other than rectangular dunelet forms. It is unlikely that the underlying idea of the mechanism of alignment can be made to account for the spacing or the height of seifs, for which the thermodynamical considerations mentioned by Brig. Bagnold (1952) seem to provide the answer.

With the material available here no real further check or advance can be made. It is therefore necessary to check on the ground or on air photos of other regions the co-existence of seifs and dunelets in their possibly diverse forms and to compute their alignments by the method suggested. In less peculiar wind regimes it will be necessary to allow for the different wind strengths and durations by the third power law before making the vectorial displacement diagram.

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Occurrence of the Plum Sawfly (Hoplocampa Flava L.) in Israel and its Control

The plum sawfly, a serious pest to plums in Israel, infests only the hilly parts of the country: the Judean Hills, the Samarian Hills, and Upper Galilee. According to Bodenheimer[1], the first adults appear in the Jerusalem area during March and the females start ovipositing one day after emergence. Incubation period lasts about 8 days. At the end of spring the larvae penetrate the soil for aestivation and hibernation. Pupation takes place the following February and emergence in March.

The extent of damage is subject to varietal as well as to yearly differences. Some of the varieties evade the damage if their flowering period comes before or after the appearance of the adults. Spraying the trees 10 days after the peak of the flight of the wasps would be most desirable according to Bodenheimer, but, on the other hand, difficulties in timing this spray led him to recommend two sprays of lead arsenate, the first when about half the petals have dropped and the second 5—10 days later. The aim of the work described below was to establish exact spraying time and to determine a more effective insecticide.

In 1950, traps with lead arsenate sugar as bait were tried as indicators for the appearance of wasps. The traps were unsatisfactory and therefore in 1951—53 a breeding box resembling that of Sprengel[2] was used and found to be very successful.

At the same time flowers were also examined under a stereoscopic microscope for egg pockets and for larvae (Table I). In 1950, the percentage of fruits on the trees infested with larvae was 86.5 on 6.4 and 57.8 on 19.4. By 27.4 the dropped fruits were free of larvae. In 1951, the percentage was 82.5 on 16.4 and 50 on 24.4. By 24.4 the dropped fruits were still infested (17.3%). Since the flowering period of the different varieties is so variable (Table II), it was impossible to time the spraying according to their phenology. The varieties Santa Rosa, Beauty, and Ogden, are heavily infested almost every year, while Kelsey, Climax, *Prunus pissardi*, and Gaviota, are usually only slightly infested and in some years not infested at all. Since the incubation period of the eggs lasts 8 days, it was decided to spray the plum trees 8 days after the appearance of the first wasps in spring. Examination of fruits and larvae has shown that the latter penetrate the soil at the age of about 4 weeks.

TABLE I

Examination of the Ogden variety during 1950-51

Date	*State of blossom*	*Percentage of flowers* *without pockets*	*pockets with eggs*	*pockets with larvae*	*empty pockets*
	1950				
9.3	Closed buds	100	0	0	0
19.3	Full bloom	100	0	0	0
21.3	1/3 of petals dropped	95	5	0	0
30.3	3/4 of petals dropped	82	5	1	12
	1951				
19.3	Beginning of bloom	99	1	0	0
26.3	Full bloom	95	2	0	3

TABLE II

Dates of plum blossoming and sawfly appearance at Kiryath-Anavim

Year	*First appearance of wasps*	*Blossoming dates* *Santa Rosa beginning*	*full*	*end*	*Beauty beginning*	*full*	*end*	*Ogden beginning*	*full*	*end*
1950	20.3	9.3	19.3	29.3	9.3	19.3	29.3	9.3	19.3	29.3
1951	15.3	11.3	19.3	2.4	18.3	26.3	2.4	18.3	26.3	16.4
1952	5.3	22.2	5.3	21.3	3.3	21.3	10.4	3.3	21.3	18.4
1953	28.3	26.3								

TABLE III

Final results of control experiments in the Ogden variety at picking time, 1950

Treatment	*Number of fruits*				*Percentage of infestation*
	Picked	*Thinned*	*Dropped*	*Total*	
D.D.T.	2490	1100	590	4180	2.8
Methoxychlor	5085	2180	410	7675	0.8
Parathion	2370	1300	433	4103	0.02
Quassia	3990	1400	275	5665	0.7
Agrocide	4950	1450	361	6761	0.09
No treatment	1620	450	906	2976	31.2

1 kg = 30 fruits.

TABLE IV

Results of control experiments in the Ogden variety, 1951

Treatment	*April 16th*			*April 24th*		
	Fruit drop		*Percentage of infestation on the tree*	*Fruit drop*		*Percentage of infestation on the tree*
	Total number	*Infested %*		*Total number*	*Infested %*	
Agrocide 3 (dusting)	0	0	0	20	0	0
Wettable Agrocide (0.2%)	0	0	0	20	0	0.3
Wettable Agrocide (0.3%)	0	0	0	20	0	0
Wettable Agrocide (0.5%)	0	0	0	20	0	0
No treatment (old tree)	30	100	9.1	30	96.7	0.6
No treatment (young trees)	50	100	49.2	25	80.0	25.0

Experiments carried out in 1950. The insecticides used were as follows: 1) a 0.5% suspension of D.D.T. 50% wettable powder*; 2) a 0.5% suspension of Methoxychlor 50% wettable powder*; 3) a 0.08% suspension of Parathion[3] 25% wettable powder**; 4) a 0.5% suspension of Agrocide wettable powder (6.5% gamma B.H.C.***); 5) Quassia prepared in the following way: 3 kg of flakes were put into water for three days: the extract was filtered before the spraying and water was added up to the amount of 100 litres.

Each of the experimental plots (at Kiryath-Anavim) was in the form of a quadrangle and contained 16—20 trees, and an untreated check plot consisted of 8 trees. The trees were sprayed by means of a power sprayer on March 29th.

Examinations were made of all the dropped fruits and of samples taken from the fruits on three trees from the centre of each experimental plot (to avoid influence of another insecticide). The fruits were counted during thinning and weighed after picking. Ogden fruits were chosen for purposes of comparison (Table III).

* Products of J. R. Geigy A. G., Switzerland.
** Product of Agricultural Chemical Co., Pittsburg, U.S.A.
*** Product of Plant Protection Ltd., London, England.

Percentage of infestation of the untreated check trees was much higher than 31%. Owing to the small number of fruits on these trees each fruit was large (1 kg contained much less than 30 fruits). Secondly, the check trees were not ideal as their peripheral parts got some of the spray, and the infestation percentage in their inner parts was really about 100.

The following conclusions can be drawn from the experiments: 1) The timing of spray in relation to the phenology of *Hoplocampa* is essential for the satisfactory control of this pest. 2) All the compounds tried gave satisfactory control of this pest. But on the other hand each has its advantages and disadvantages: D.D.T., and to a lesser extent Methoxychlor, proved to be less effective than others. Parathion gave excellent control, but owing to the high toxicity of this material to human beings it was not recommended. Quassia gave similar results to that of Methoxychlor, but owing to its high price and difficulties of preparation it was not recommended. Agrocide was almost as effective as Parathion and its taint disappears by picking time since spraying is carried out early in the season. Consequently Agrocide was recommended.

Experiments carried out in 1951. Agrocide wettable powder in different percentages and Agrocide 3 powder (0.45% gamma B.H.C.) were used. Each plot contained 25 trees of both Ogden and Santa Rosa. The spraying and the dusting took place at Kiryath-Anavim, on March 26th. 300 Ogden fruits on the trees and all the dropped fruit were examined. An old tree of Ogden variety and a plot of young trees were chosen as an untreated check, but the first got some spray on one of its sides and so could not be considered as an ideal check.

Table IV shows that the percentage of infestation in the treated trees was negligible. Agrocide wettable powder secured satisfactory results even at a concentration of only 0.3%. Dusting with Agrocide 3 powder also proved to be effective enough.

15 mm of rain which fell two days after the treatment did not reduce the effectiveness of control. No evidence has yet been obtained, however, as regards the washing off effect of heavier rains than that.

The fruits from experimental and check trees were tasted by different people and no difference was found by them. The bee-hives in Kiryath-Anavim suffered heavy losses from the spraying and even more so from the dusting.

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The Development of Gonads in Blaps cribrosa Sol. and B. tenuicollis Sol.

The female reproductive system consists of 2 ovaries, each divided into 6 or 7 ovarial sacs; each ovarial sac is topped with 8 to 10 acrotrophic ovarioles ending in germarium with terminal filaments. All the mature eggs pass on to the ovarial sac which holds a maximum of 20 eggs. The total number of eggs, therefore, in two ovaries is between 250 and 300 per season. The receptaculum seminis is tubular and branches out from the lower part of the oviduct; its tip ends in the accessory gland which is a long irregularly coiled tubular organ covered with masses of fat. In December and January the size of the ovary is smallest, with an average length of 5 mm, and the largest egg measures approximately 0.5 mm. During February, March and April, the ovary gradually increases in size, and towards the end of May it reaches its maximum size, and oviposition starts at about the same time. From June to the end of October the ovary is the largest and the egg reaches its maximum length of 2.5 mm. All through this period, *Blaps* mate and oviposit. At the beginning of November the ovary shrinks to 7 mm in length and 6 mm in width. At the time of emergence the ovary measures about 4 × 3 mm and contains a few small, yellowish eggs, approximately 0.4 mm long. The germarium, too, is yellowish but, as they grow, both eggs and germarium turn whitish. *B. cribrosa* and *B. tenuicollis* become sexually mature approximately 3.5 months after emergence. When the eggs are fully mature, only a few descend the oviduct at one time, thus explaining the long period of oviposition lasting from the end of May to the end of October. No more eggs mature after May.

The male reproductive system consists of 2 follicular testes, a thickened vesiculum seminalis, two pairs of accessory glands: mesadenes and ectadenes, and a ductus ejaculatorium which is long and convoluted. The monthly fluctuation of the testis of *B. cribrosa* and *B. tenuicollis* is as follows: in January the testis is smallest; from February to June a gradual increase is observed, and during July, August, September and October, it reaches its maximum size of 7 mm. Then in November it shrinks to 5 mm. However, mature spermatozoa are observed all through the year although the number fluctuates according to the size of the testis. Histologically, the development of the testis may be divided into 4 periods: in the first period (December/January) the cross section of a follicle shows that most of the cells contain spermatogonia and beginnings of spermatocytes. Only a few cells on the outer margin of a follicle are differentiated, and spermatids are seen scattered all over the cells (Figure 1). In the second period (March—April) the cross section of a follicle reveals differentiated cells all along the margin and undifferentiated ones in the

Figures 1—5

middle of the follicle, with many mitoses. Most of the differentiated cells indicate the concentration of spermatids either on one or on both poles while in few cells; this concentration is completed forming a conspicuous, dark, beak-like projection on one or both sides of the cell (Figure 2). Further advance is observed in the third period (June—July), in which there are only 2 kinds of cells: about one half of the follicle consists of beaked cells described above and in the rest of the half the sharp points of concentration are broken, setting the mature spermatozoa free into the cavity of the follicle, as shown in Figure 3. June—July is the period of mating and oviposition, and the size of the testis reaches its maximum of 7 to 7.5 mm. In the fourth period (August—October) all the follicles are in the same state, discharging spermatozoa. This period marks the peak of the sexual activity, with each follicle teeming with actively moving spermatozoa (Figure 4). In newly emerged males, the testis measures about 4 mm and the cross section of a follicle reveals the zones of spermatogonia (Figure 5). It takes 3 to 4 months until the male is sexually mature and starts copulation. The adult *Blaps* may live for a few years during which ovaries and testes repeat the seasonal fluctuation started above.

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Changes of Serum Total Lipids, Total Cholesterol and Lipid-Phosphorus in Jewish Yemenite Immigrants after 20 Years in Israel+

As is known to clinicians in Israel, cases of myocardial infarction or other manifestations of atherosclerosis are very rare among Jews of Yemenite origin who form about 6 % of Israel's total population. Dreyfuss[1] reported two cases of myocardial infarction among Yemenite Jews, but notes that one of the patients was living in Israel for 35 years and the second for 21 years. Among the 650 cases of myocardial infarction admitted to the Beilinson Hospital in the period 1936-1954, none were of Yemenite origin. However, one Yemenite Jew admitted to our hospital, suffered from generalised atherosclerosis. This patient had been living in Israel for 38 years.

Following the general idea that there exists a direct relation between the concentration of the serum cholesterol and the development of atherosclerosis[2,3], we determined the values of serum cholesterol, as well as serum total lipids and lipid-phosphorus in two groups of Yemenite Jews: those living in Israel for 20 years or more (old immigrants) and those who came to the country 3-5 years ago (new immigrants).

We have so far examined 75 persons from the old immigrants group (34 men and 41 women) and 192 persons of the new immigrants group (115 men and 72 women). For obvious reasons we have chosen the 35-65 age groups.

We have included in this study only persons who on clinical and laboratory examinations did not show any signs of disease. Laboratory examination of stools were carried out in many of these cases so as to exclude persons suffering from parasitic diseases. Persons with enlargement of liver and spleen over 3 cm were excluded as well. No cases of clinical undernourishment were included although a considerable number of individuals in the new immigrant group were very thin.

Samples of blood to be examined were taken 12 hours after the last meal. Each determination was made twice. No values of cholesterol with a difference of more than 5 % were accepted.

All the biochemical determinations in this study were carried out by one person (D.A.) so that the error due to the examination itself is probably diminished.

Methods of Biochemical Determinations

Total cholesterol: according to Sackett's modification of Bloor's method.

Lipid phosphorus: according to an adaptation based on the methods and modifications of Fiske and Subbarow, Bloor, Walker and Huntsinger, and Roe and Whitmore.

Total lipids: according to the turbidimetric method of Kunkel, Alvens and Eisenmeyer.

+ With the aid of a grant from the Central Office of the Sick Fund of the General Federation of Jewish Labour in Israel.

TABLE I (Mean values)

Yemenite Immigrants	Age Group	Number of cases	Height (cm)	Weight (kg)	Hb. (g)	Total Lipids (mg %)	Total Cholesterol			Lipid-phosphorus (mg %)	Ratio Chol. mg% / Lip. mg%	Phospho-Lipids (mg %)	Blood Pressure
							Mean value (mg %)	S.D.***	C. V. (mg %)****				
A. MEN													
Old*	35-44	13	166	64.6	13.8	640	188	36.4	19.4	9.3	20.5	232	125/75
New**	35-44	49	167	63.0	13.6	558	146	28.4	19.5	8.4	17.9	210	120/70
Old	45-54	15	164	60.3	14.1	695	195	34.4	17.6	9.6	20.4	240	125/75
New	45-54	43	164	61.5	13.6	602	160	31.1	19.4	8.9	18.6	222	115/65
Old	55-64	6	160	56.2	14.0	654	191	41.9	21.9	10.4	18.6	260	140/75
New	55-64	23	168	61.3	13.1	603	158	33.7	21.3	9.5	17.7	227	125/70
B. WOMEN													
Old	35-44	20	152	51.5	12.1	688	196	26.1	13.3	9.8	20.2	243	125/80
New	35-44	39	156	52.7	12.6	620	172	25.9	15.0	9.8	18.5	240	115/70
Old	45-54	16	152	60.1	12.5	731	213	23.6	11.1	10.6	20.7	264	130/80
New	45-54	30	156	53.1	12.4	607	170	30.8	18.0	8.7	19.8	219	120/70
Old	55-64	5	152	54.0	13.3	732	220	33.2	15.0	10.9	20.3	272	150/85
New	55-64	8	155	56.3	12.7	615	205	23.0	11.2	10.3	20.8	255	130/75

* Living in Israel over 20 years; ** Living in Israel 3—5 years;*** S.D. — Standard of Deviation;**** C.V. — Coefficient of Variation.

TABLE II

*Comparison of serum lipids, cholesterol and lipid-phosphorus in the old and new Yemenite immigrants**

e up.	Total lipids		Relative Difference %	Total cholesterol		Relative Difference %	Lipid-phosphorus		Relative Difference %
	Old Immig.	New Immig.		Old Immig.	New Immig.		Old Immig.	New Immig.	
MEN									
-44	639	558	11	188	146	28	9.3	8.4	10.0
-54	695	602	15	195	160	22	9.6	8.9	7.8
-64	654	603	8	191	158	20	10.4	9.5	9.8
WOMEN									
-44	688	620	11	196	172	14	9.8	9.8	0
-54	731	607	20	213	170	22	10.6	8.7	21.0
-64	732	615	19	220	205	7	10.9	10.3	0.3

*or the purpose of this Table values of the new mmigrants' group were considered as 100%.

The analysis of our findings shows that, the erage weight of the men examined, especially the new immigrant group, was somewhat lower n the standard, while the average weight of the men in the age-groups 45-64 years tended to rease. The average blood pressure of the old migrants was low, while the blood pressure of new immigrants was even lower.

The average hemoglobin value did not differ m the generally accepted average values in ael.

Serum total lipids was within the limits of the rmal[4], but in all the age groups the serum total ds of the old immigrants was 8-20% higher n the serum total lipids of the new immigrants. e same difference existed in both sexes.

The values of serum total cholesterol in the *old migrants* was lower than the accepted values in European Jews in Israel as well as lower than ues reported by Keys[5] and Page[6] for the USA, close to those found by Keys[7] for Italy, Spain l South America. The serum total cholesterol *new immigrants* was even lower (7-28%) than t in old immigrants.

The values of serum lipid-phosphorus, as those serum cholesterol, were higher in the old immi- nt group than in the new immigrants and were nd to be shifting towards the higher values epted as normal for European Jews in Israel.

The higher concentrations of serum cholesterol lipid phosphorus in old immigrants, which y explain the beginning of appearance of erosclerosis in this group, might be due to a her standard of living and the new dietary ha- which they adopt during their long stay in ael.

t is interesting to note that the biochemical va- s in women of both groups is somewhat higher n in men, although their nutrition is similar to t of the men examined.

In cases which showed very low values of serum total cholesterol, liver function tests were performed. Out of 63 liver function tests, 9 cases (i.e. 14.3%) were found to be positive. These cases were not included in our data; therefore we assume that the low serum biochemical values are the result of long standing dietary deficiency. This may be the reason that no clinical signs of atherosclerosis are found among the new Yemenite immigrants, for it is well known that dietary deficiency reduces the incidence of atherosclerotic processes[8].

We have not yet completed the collection of enough data concerning diet (including the percentage of fats in the total caloric intake) and dietary habits of new immigrants.

This is a preliminary report. An additional number of persons of both Yemenite groups is being examined, after which studies of cholesterol metabolism in both groups will be made.

We gratefully acknowledge the contribution of Professor A. Katchalsky of the Weizmann Institute of Science, Rehovot.

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TH MEETING OF THE MICROBIOLOGICAL SOCIETY

MARCH 17—19, 1954, JERUSALEM

CONTENTS

Symposium on the Standardization and Unification of Serological Diagnosis and on Countrywid Cooperation in Epidemiological Work *

The Development of Standardization of Agglutination Tests, A. L. OLITZKI, *Hebrew University - Hadassah Medical School, Jerusalem.*

Standardized Antigens for the Widal Test, W. SILBERSTEIN, *Government Central Laboratories, Ministry of Health, Jerusalem.*

Standardization of Vi-Antigens, E. EYLAN, *Epidemiological Laboratories, Ministry of Health, Tel Aviv.*

Standardization of the Brucella Agglutinatio Test, D. SULITZEANU, *Hebrew University - Ha dassah Medical School, Jerusalem.*

The Epidemiological Significance of Phage Typ of Salmonella typhi and Salmonell paratyphi B, E. EYLAN, *Epidemiological La boratories, Ministry of Health, Tel Aviv.*

Final Agreement Regarding Laboratory Procedures and Countrywide Cooperation

(a) *Standardization and Unification of Serological Diagnosis*

Diagnostic laboratories are advised to use the following standard suspensions:

1) *H-* and *O*-suspensions for diagnosis of enteric fevers from the Government Central Laboratories, Jerusalem (Director, Dr. W. Silberstein).

2) Vi-suspensions for detection of typhoid carriers and *Proteus* suspensions for diagnosis of rickettsiasis from the Epidemiological Laboratories, Tel Aviv (Director, Dr. E. Eylan).

3) *Brucella* antigen from the Veterinary Labo ratory, Tel Aviv (Director, Dr. J. van der Hoede

(b) *Improvement and Cooperation in Epidemiol gical Work*

1) All freshly isolated *Salmonellae* should b sent to the Government Central Laboratories, J rusalem, for final serological identification.

2) All strains of *S. typhi* and *S. paratyphi* should be sent to the Epidemiological Laborat ries, Tel Aviv, for phage typing.

Intestinal Bacteria

On the Antigenic Relationship between Salmonella and Shigella Species, E. EYLAN, *Epidemiological Laboratories, Ministry of Health, Tel Aviv.*

Salmonella in Tortoises, W. HIRSCH and RAYA SHAPIRO-HIRSCH, *Central Laboratory, Kupat Holim, Haifa.*

1954, *Harefuah*, **46**, 237.

On the Incidence of Salmonellosis and Shigellosis, P. LUBLING, *Kupat Holim, Tel Aviv-Jaffa.* This summary covers stool specimens submitted for examination at the Kupat Holim Central Laboratory, Tel Aviv, during 1951—53. Specimens were taken from a cross sampling of approximately 300,000 people.

Year	*No. of stools examined*	*No. positive* Salmonella *and* Shigella
1951	11,538	1,197
1952	9,605	1,258
1953	8,146	1,117

The monthly summary of the findings show that the dormant months were January, Februar March and April.

About 20% of the positive findings were *Sa monella* strains, among which 27 serotypes we isolated during July, 1952 — December, 1953 In a total of 382 strains, 20% were *S. typhi* an *S. paratyphi* A, B and C.

* Strains kindly confirmed and in the majority of cases ide tified by the Salmonella Ref. Lab., Jerusalem (Dr. Silberste and Dr. Gerichter).

* 1954, *Harefuah*, in press.

In the remaining 80%, *S. typhi murium* compri-
d about 35% of the cases, *S. newport* 12.6%,
meleagridis 6.3%, *S. branderup* 4.7% and *S.*
nteritidis 4.2%.

During this period two new serotypes were
olated: *S. hadar*[1] and *S. nachshonim*[2]. This type
ntains antigen XXXVI, which is not expressed
its diagnostic formula.

Among the Shigellas, *Shigella dysenteriae* Type I
appeared after many years of absence, with 6
ses in 1951, 13 cases in 1952 and 9 cases in 1953.
different seasonal peak was observed between
. sonnei and *Sh. flexneri*, the former in the begin-
ng of summer and the latter in autumn. Be-
nning with the typing of the *Sh. flexneri* strains in
52 (460 cases), we found the prevailing strain was
. flexneri II (W) with 170 cases (37%); next
me *Sh. flexneri* III (Z) with 124 cases (26%) and
. flexneri VI (Boyd 88) with 88 cases (19%).

The table below shows the results for 1953 (total
ses 457).

pe	I	II	III	IV	V	VI	VII	IX	X	XI	XIV
ses	1	92	56	28	3	151	1	33	4	6	82

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KAUFMANN, F., SILBERSTEIN, W. and LUBLING, P., 1953. *Acta Pathol. et Microb. Scandinavica*, 33, 79.

almonella Findings in Israel, W. SILBER-
EIN, *Government Central Laboratories, Ministry*
Health, Jerusalem. During 1952—54, 939 strains
re sent to the National Salmonella Centre of
rael for identification, as opposed to 319 strains
nt during 1950—52.

In these 939 strains belonging to 38 different
pes, three new ones were isolated: *S. hadar, S.*
chshonim and *S. mishmar-haemek*. In addition
the new types, other types appeared in the
untry for the first time: *S. tennessee, S. brede-*
y, S. izumu, S. canastel, S. wichita, S. cubana,
minnesota, S. johannesburg, and *S. hessarek*.
ree types, *S. oranienburg, S. manhattan* and *S.*
adon, were found in 1952—54 but not during
e previous two years. It was also observed
at several previously present types disappeared
ile other types were repeatedly found in the
untry.

The following frequency percentage changes
ould be especially noted:

	1952-54	1950-52
typhi murium	25%	25%
newport	9%	14%
montevideo	7%	1%
meleagridis	9%	4%
enteritidis	4%	2%

The total of all serotypes reported hitherto in
ael is now 56.

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The Survival of Coliforms, Streptococcus faecalis and Salmonella tennessee, in Soil and Climate of Israel, SONYA BERGNER-RABINOWITZ, *Sewage Research Laboratory, Government Central Laboratories, Ministry of Health, Jerusalem.* The purpose of this investigation is to study the survival of coliforms, *Str. faecalis* and *Sal. tennessee*, in the soil, under the field conditions obtaining in Jerusalem. The survival of these species was studied at the soil surface and at a depth, in summer and in winter, in cultivated and uncultivated plots. The survival of *S. tennessee* was also studied in open storage tanks of the type commonly used in irrigation practice in Israel.

The results obtained from the experiments described above show a big initial reduction in numbers of both genera in *terra-rossa* soil irrigated once with sewage to which a *Salmonella* suspension was added. *Salmonella* organisms continued to decrease throughout the experiment, disappearing by the 46th day at the surface and the 70th day in depth. The coliform population, however, decreased until it became relatively stable, showing but little change with time.

A study similar to that described above, but including *Str. faecalis*, was carried out in summer. A suspension of *S. tennessee* was added to raw sewage kept in open storage tanks in the field. A portion of the mixture was applied to uncultivated plots, the rest remaining in the tanks. *Salmonella* disappeared from the surface of the soil by the 15th day, and from the depth by the 11th day. Coliforms and streptococci persisted throughout the experiment which lasted 38 days. *Salmonella* survived in the storage tanks longer than in the soil, disappearing by the 22nd day.

A study of *S. tennessee* carried out in summer in the soil of a growing sunflower plot under sewage irrigation, showed the survival time of this organism to be 23 days at the surface and 37 days in depth.

There is a straight-line relationship between the decrease in numbers of *Salmonella*, coliforms, and streptococci. The coefficients of correlation have been found to be positive and significant.

Judging by the rate of disappearance of coliforms and streptococci from soil, the latter do not appear to be preferable to coliforms as an indicator of faecal pollution. This observation seems to be confirmed by additional experiments carried

out with the same organisms as above in different media, namely raw sewage, sterilized sewage, and ordinary tap water. This study is to be continued.

Results of Blood Cultures from Patients Hospitalized in Sarafand Government Hospital, D. SAMPOLINSKY, *Assaf Harofe Hospital, Sarafand.* Of 7757 blood cultures examined during 1950—53, 653 were positive, among 40% with *Salmonella typhosa*, 9% with *S. paratyphi* A, more than 4% with *S. paratyphi* B, 10% with other *Enterobacteriacea*, 23% *M. pyogenes*, 3% beta-hemolytic, 6% alpha-hemolytic streptococci.

S. typhosa was generally found in one of the first three cultures taken from the patient. In subacute bacterial endocarditis, on the other hand the causative agent was often isolated after re peated examinations, in one case only in th eighteenth culture.

Of 125 patients with *S. typhosa*, 6 were belo the age of one year, 63 belonged to the age clas 1—5, 9 to the age class 6, and 14 to the age clas 7—14.

The opposite was seen in *S. paratyphi* A. C 16 patients, 15 were over 18 years old. In most c the patients from whom *Enterobacteriacea* othe than *Salmonella* were isolated, it was possible t show that the infection was secondary to a foca disease, the commonest place being the urinar tract.

Symposium on Poliomyelitis

Isolation and Identification of Strains of Poliomyelitis Virus in Tissue Cultures, H. BERNKOPF, *Hebrew University — Hadassah Medical School, Jerusalem*, 1954, *Harefuah*, **47**, 203.

On the Problems of Immunization and the Epidemiology of Poliomyelitis, N. GOLDBLUM, *Medical Research Laboratories, Army Medical Corps, Israel Defence Forces.*

Problems of Passive and Active Inoculation Against Poliomyelitis, E. EYLAN, *Epidemiological Laboratories, Ministry of Health, Tel Aviv.*

Viruses and Rickettsiae

Some Aspects of the Growth of Western Equine Encephalomyelitis and West Nile Viruses in Embryonated Eggs, J. FENDRICH, Y. NIR and R. GOLDWASSER, *Israeli Institute for Biological Research, Ness Ziona.*

1. *Western Equine Encephalomyelitis Virus*

This virus killed chick embryos within twenty-four hours after being injected directly into the blood steam. Samples of blood were taken periodically after infection and the titre of virus in each sample was ascertained in order to determine the characteristics of its growth curves. It was found that, immediately after infection, the virus disappeared from the blood for a period of three to three and a half hours. Variations in the size of the inoculum had no effect on the length of this period.

Growth curves following inocula ranging from 2 LD_{50} to 2 x 10^5 LD_{50} were determined. In all cases, the titre of virus found in blood increased with time, reaching a peak which was roughly the same for all inocula, the time required being much shorter following a large inoculum than a small one.

In another series of experiments, the susceptibility of chick embryos to the W.E.E. virus was investigated. The embryos were inoculated with the virus via various routes. Intravenous inoculation was more effective than either the yolk-sac or the chorio-allantoic routes. No variations in susceptibility were exhibited by embryos of different ages.

2. *West Nile Virus*

Chick embryos responded much more slowly to intravenous inoculation with this virus, the day of death varying from the third to the sixth accor ding to the size of the inoculum. The interval be tween inoculation and the reappearance of th virus in the blood appeared to be inversely pro portional to the amount of virus inoculated. Ma ximum titres of virus in blood were found onl 36 to 48 hours after inoculation. Growth curve showed much longer constant periods, slowe rates of multiplication and lower final titres tha in the case of W.E.E. virus.

As with the W.E.E. virus, chick embryos were more susceptible to intravenous than to othe routes of inoculation, the age factor being negli gible.

Studies in the Natural History of West Nile Fever 1. A Preliminary Report on the Investigations of the 1953 outbreaks of West Nile Fever, N. GOLDBLUM, V. V. STERK and WANDA JASINSKA-KLINGBERG *Medical Research Laboratories, Army Medical Corps, Israel Defence Forces.* During the outbreaks of West Nile Fever (WNF) in the summer of 1953, a large group of patients was examined for the presence of virus in the blood and in many of them the development of antibodies was followed. A number of apparently healthy individuals from the epidemic areas was included in this study. From 208 patients, diagnosed as having WNF or "Fever of unknown origin", virus was isolated by intracerebral inoculation of the acute-phase sera in adult mice, in 42 instances. Virus was found in the blood of 4 healthy individuals out of 306 examined. The isolated strains were passaged three times intracerebrally in mice, and then identified by intracerebral challenge of ham-

sters hyperimmunized to a known strain of West Nile virus. Thus, 25 of the virus strains isolated have been identified as West Nile virus; the others are being tested in the same way. In collaboration with Dr. K. Marberg, of the Assaf Harofe Government Hospital, a detailed study was made on a selected group of 74 patients. In some of them, virus was found in the blood on two to three consecutive days during the 1st to the 4th days of illness. Findings in other patients indicate that virus is also present in the blood during the pre-clinical stage. Complement fixing (c-f) and neutralizing antibodies to West Nile virus were followed in the majority of the patients. A rise in c-f antibodies, from less than 1:2 to 1:32—1:256, usually occurs during the 2nd week of the disease, and the titre attained remains practically unchanged for at least four months (the longest time tested). Neutralizing antibodies apparently develop at a somewhat later date and rise steadily thereafter during 2—3 months. In addition to homologous antibodies, the development of antibodies to Japanese Encephalitis virus (JE) was tested in certain groups of patients. Antibodies to JE develop during convalescence in more than 50 percent of the patients but to a somewhat lower titre. The significance of these findings and the possibilities of their practical application will be discussed.

Studies in the Natural History of West Nile Fever. 2. Transmission of West Nile Virus by Laboratory-bred Culex mosquitoes, V. V. STERK, A. S. TAHORI and N. GOLDBLUM, *Medical Research Laboratories, Army Medical Corps, Israel Defence Forces.* Successful transmission of West Nile virus by laboratory-bred *Culex molestus* to infant mice is reported. The mosquitoes were fed a mixture of virus suspended in saline and defibrinated normal rabbit blood, soaked on cotton. They were then kept at a temperature of 28° C for the duration of the experiment. In each experiment, virus with a different mouse intracerebral titre, ranging from $10^{-6.5}$ to $10^{-2.8}$ was used. At different time intervals, from 7 to 28 days following the infectious meal, infant mice were introduced into the cages holding groups of infected mosquitoes. At the same time, titrations were made on suspensions of triturated mosquitoes. It was found that, although virus was present in appreciable titre in the mosquito suspensions on days 7—28 following the infectious meal, the mosquitoes could transmit the virus by bite to infant mice only after the 12th day. Infection of infant mice by the bite of infected mosquitoes was obtained on days 12, 14, 15 and 21 after the infectious meal. Further experiments are being done in order to determine whether virus can be transmitted after the 21st day. Experiments to transmit West Nile virus from infected to healthy hamsters by the bite of laboratory-bred *Culex molestus* are in progress.

The Susceptibility of the Golden Hamster to Semliki Forest Virus, A. MICHAEL DAVIES, *Department of Hygiene, Hebrew University—Hadassah Medical School,* J. FENDRICH, and Y. NIR, *Israeli Institute for Biological Research, Ness Ziona,* and YONA YOSHPE-PURER *Epidemiological Laboratories, Ministry of Health, Tel Aviv-Jaffa.* The golden hamster has been shown to be susceptible to Semliki Forest virus administered intracerebrally, intraperitoneally, intranasally and subcutaneously, but not intragastrically or intrarectally. The LD_{50} dose is equivalent to 30 mouse LD_{50} doses when estimated by intracerebral injection, 300 doses when given intraperitoneally and to up to 1000 doses subcutaneously. There was very considerable difference in the susceptibility of individual animals to virus administered peripherally. No differences in reaction were noted towards egg, mouse and hamster passaged virus material. Clinically, the first sign was paralysis of one or both hind limbs 4—6 days after infection, which was followed by quadriplegia, moribundity and death 1—2 days later. On several occasions animals recovered completely from paralysis consequent on the administration of 10—100 lethal doses intraperitoneally.

Histologically, moribund animals showed lesions confined to the central nervous system consisting of diffuse cellular infiltration and moderate neurophagia. Many large ganglion cells of the brain and spinal cord showed degeneration, as did Purkinje cells of the cerebellum.

A group of month old hamsters was infected intraperitoneally with 10,000 LD_{50} doses and daily titrations were made of pools of brains and bloods. Virus content of the blood showed a peak after 24 hours, falling to zero after 4 days, while the titre in the brain rose slowlyto a maximum plateau after 3 days, falling gradually after the 6th day. By the 6th day 25% of the animals showed signs of paralysis.

Epidemiological Observations on Ornithosis in Israel, A. ELIZUR and H. BERNKOPF, *Hebrew University—Hadassah Medical School, Jerusalem.* Viruses of the psittacosis lymphogranuloma venereum group were isolated from four cases of atypical pneumonia in very young children. In two cases which ended fatally, virus was isolated from autopsy material and in two other cases from the blood. In three cases sera obtained from the patients' parents showed a significant titre of complement fixing antibodies. In one of these cases the mother reported to have suffered from a severe cough before the child's illness.

Serological tests were carried out with the sera of 50 cases of atypical pneumonia in children. Both the complement fixation and the haemagglutination inhibition test were employed. 12% of the cases showed significant titres with both tests. Two cases showed a positive haemagglutination inhibition test only.

Over a hundred pigeons from several lofts in Jerusalem were tested for antibodies using both tests. 25% were found positive with the complement fixation and 60% with the haemagglutination inhibition test. The possible epidemiological implications of these findings were discussed.

Survey of the History of Murinie Typhus in the Tel Aviv Area, E. EYLAN, *Epidemiological Laboratories, Ministry of Health, Tel Aviv.*

Attempt to Transfer the West Nile Virus in Culex Molestus, E. EYLAN and S. DAVIDOVITCH, *Epidemiological Laboratories, Ministry of Health, Tel Aviv.*

The Transmission of Semliki Forest Virus by Aedes Aegypti, A. MICHAEL DAVIES *Department of Hygiene, Hebrew University—Hadassah Medical School, Jerusalem,* and YONA YOSHPE-PURER, *Epidemiological Laboratories, Ministry of Health, Tel Aviv.* A large batch of *Aedes aegypti* mosquitoes was fed on a mixture of defibrinated rabbit blood, honey and virus containing mouse brain, after a preliminary period of starvation. Small numbers were removed from the cage and allowed to feed on mice at various periods after infection. Positive transmission of the disease was obtained after 9 and 12 days but not after 13 and 32 days. Mosquitoes triturated and injected into mice did however produce the characteristic disease pattern after 32 days, showing persistence of the virus. Attempts to perform serial passage of the virus in mosquitoes were not successful.

10 day old embryonated eggs were infected intra-allantoically with mouse brain virus material and at the peak of the viraemia (16—18 hours) mosquitoes were allowed to feed through the exposed egg shell membrane. The next day and subsequently, groups of 20 mosquitoes were removed from the cage, triturated and titrated for their virus content by injection into mice. Other groups were allowed to bite mice daily. The virus content of the mosquito pools fell from an initial level of 10^5 mouse LD_{50} doses/0.1 ml to $10^{2\cdot5}$ doses after 24 hours, with a subsequent rise to 10^4 on the 4th day and a maximum of 10^5 on the 10th day. Transmission of the virus by biting was successful only between the 6th and 9th days, utilising between 3 and 7 *Aedes*. In other experiments with initial infecting doses varying from 10^4 to $10^{6\cdot5}$ LD_{50}, the initial drop of virus content was always observed, but in no case was there a significant subsequent increase above the level of infection. Ability to transmit the virus was related to the time of the peak of the curve of virus content, but not to the absolute level of virus. No differences were observed between batches of mosquitoes infected with mouse, hamster and egg passaged material. Transovarial passage of the virus did not occur.

It is suggested that *Aedes aegypti* can act only as a sporadic transmitter of Semliki Forest virus and, as there is no multiplication of the virus in the body of the mosquito, it is probably not of great epidemiological significance as a potential vector.

(These investigations were carried out at the Israeli Institute for Biological Research, Ness Ziona).

General and Applied Bacteriology

Loss of the Ability of Acetobacter xylinum to Synthesize Cellulose, M. SCHRAMM and S. HESTRIN, *Hebrew University—Hadassah Medical School, Jerusalem.*

Observations on the Deterioration of Fishing Nets in Israel, M. ASCHNER and Z. ELIASH, *Department of Bacteriology, Hebrew University - Hadassah Medical School, Jerusalem.* The fishing nets used here are usually made of cotton or flax, i.e. of cellulose fibres. As treated at present, they deteriorate rather quickly, involving of course a considerable loss of foreign currency.

We studied the process of disintegration of the nets in fresh and sea water, and found that bacteria, not fungi, are responsible for the damage. Several species of bacteria were isolated with the aid of "xylinum membranes"[1]. Two have been identified so far as *Cellvibrio vulgaris* and *Nocardia maculata.*

It was desirable to use a method permitting a comparison of the various known preservation techniques in the shortest time possible. After various trials we found that the quickest results were received by placing the material to be tested on moist garden soil. In this way results could be read after two weeks. It was found that the method generally employed here — treatment with cutch only — is not very efficient. More elaborate methods, in which the tanning component of the cutch is made insoluble by an aftertreatment with metal compounds, consistently gave better results. This result was verified with flax yarns immersed in the sea for periods up to six months.

A grant for this research from the Department of Fisheries of Hamashbir Hamerkazi is herewith gratefully acknowledged.

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The Yeast-Flora of Rats on Normal Diets and on Diets Supplemented with Antibiotics, M. ASCHNER, S. HALEVY and DINAH AWRAM, *Dept. of Bacteriology and Lab. of Nutrition, Hebrew University—Hadassah Medical School, Jerusalem.* Yeasts, among other microorganisms, are regular components of the intestinal contents of rats. Very little is known about the taxonomic position and the ecological role of these microorganisms in the rat.

In a study of the microbial flora of rats which received in their diet additions of various anti-

biotics, we attempted to evaluate the changes brought about by these supplements in the yeast population of the rat cecum.

The rats were sacrificed after being kept for about 5 weeks on a diet supplemented with 50 μg penicillin or aureomycin per kg diet. The cecum was aseptically removed and its contents were diluted 1:100 with saline. The material was then homogenised in the Waring Blendor and different dilutions plated out on Difco-Malt agar to which penicillin and streptomycin were added to bring the concentration of each antibiotic to about 10 μg per ml. This concentration inhibited the bacteria but had no effect on the yeasts, so that only yeast colonies developed on these plates.

In this way it was found that one g of fresh cecum content contains about 10 million live yeast cells. The number is probably still greater as microscopic examination of the cecum contents reveals the presence of many cell groups. This result is the average of determinations made from 12 rats. There was no great difference among the individual rats.

The number of yeast cells was also determined in rats which were kept on a normal diet, free of antibiotics. An average of 20 determinations gave one million cells (10 times lower than in the rats with the supplemented diet).

The number of yeast types encountered was rather small. Usually all the colonies in the highest dilution plate belonged to one and the same species. This species, which occurred practically in all the rats examined, corresponds in its taxonomic characteristics to *Torulopsis glabrata*. It differs however from this species in that its growth on agar media is very poor.

This weak growth outside the rat cecum indicates that the yeast in question is not a chance invader of the rat intestine but a component of its permanent microbial flora. A few species of the genus *Candida* were also found but less regularly and always in much smaller numbers.

Levan Formation in a Species of Corynebacterium. J. Henis and M. Aschner, *Dept. of Bacteriology, Hebrew University-Hadassah Medical School, Jerusalem.* The levan producing bacterium discussed here appeared as an air contamination. It was found to grow characteristically on a simple medium containing agar-agar, tap water and 10% sucrose. The colonies are large, viscous, transparent, and have a typical glossy, bead-like appearance. They are able to rise from a base of 6 mm to a height of 7 mm or more. There is no pigment formation. The organism grows also on standard or glucose yeast extract agar. On this medium, however, colonies are flat and coloured by a yellow-orange pigment. Microscopic examination of these colonies reveals short, non-motile, gram positive rods. Their appearance was found to be regular after 18 hours, and coccoid after 48—72 hours. According to its morphological properties, this bacterium probably belongs to the genus *Corynebacterium.**

The list of levan producing bacteria includes species belonging to many different genera and families, such as *Bacillaceae*[1], *Enterobacteriaceae*[2], *Pseudomonadaceae*[3], *Azotobacteriaceae*[4]. No levan producing species of *Corynebacterium* has so far been reported.

The nature of the capsular substances

The capsular substance formed by this bacterium on a sucrose-containing medium is precipitated in 70% ethanol, does not reduce Fehling solution, and is completely hydrolysed by 0.5% oxalic acid after one hour heating at boiling temperature. It is also precipitated by an antiserum effective against levan of *A. levanicum*. Examination by a chromatographic method reveals the presence of fructose without any other sugar noticeable.

Deposition of levan in the culture medium

If bacteria washed from a standard agar slant are suspended in a 10% solution of sucrose in tap water, a gel-like deposit forms at the bottom of the test tube, which is visible macroscopically already after 6—7 hours and is almost fully developed after 24 hours.

Optimum conditions for the formation of this sediment are: a temperature of 30 °C, a slightly alkaline pH, concentration of about $4 \cdot 10^8$ cells per cc, and a slanting position of the test tube, which allows for good aeration. Under such conditions nearly half of the culture fluid is taken up by the newly formed gel which contains about 5% levan. The supernatant is clear, with practically no levan. Microscopic examination indicates that the gel is formed by sedimentation and coalescence of single clumps of bacteria surrounded by masses of extracellular polysaccharide at the bottom of the test tube.

Effect of number of organisms on levan formation

The number of bacteria present in the sucrose solution affects the maximum formation of levan in the following way: at the concentration of $4 \cdot 10^8$ cells/cc, after two days levan equivalent to 27 mg fructose per cc is formed from the 10% solution. If the number of bacteria is lower or higher, the total amount of levan formed decreases.

Effect of peptone concentration

The presence of 1% peptone in the sucrose solution affects levan formation adversely, levan being formed at about one third the amount formed under optimal conditions.

A more detailed account of the behaviour of this organism under different conditions will be given elsewhere.

* A more detailed description of this organism and discussion of its taxonomic position will be given elsewhere

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Pyocyanine Synthesis by Pseudomonas aeruginosa, Y. S. HALPERN and N. GROSSOWICZ, *Department of Bacteriology, Hebrew University - Hadassah Medical School, Jerusalem.* Pyocyanine, the blue pigment formed by *Pseudomonas aeruginosa.* is endowed with important biological properties: it possesses antibiotic activity against gram-positive microorganisms; it may participate in oxido-reduction systems, etc. While publications by other workers on pyocyanine formation were confined to growing cultures, the object of the present communication is to provide information on pyocyanine production by washed suspensions of *Ps. aeruginosa*, dissociated from bacterial growth.

Among various amino-acids tested, glutamic acid was found to be the best substrate for pyocyanine formation, serving as the sole carbon and nitrogen source. Other compounds able to replace glutamic acid were asparagine, aspartic acid, glutamine, proline, arginine, histidine and alanine, and organic acids as succinate,fumarate and pyruvate, in presence of ammonium ions. No pyocyanine was formed with other amino-acids (glycine, leucine valine, phenyl-alanine, threonine, lysine, methionine and cysteine), nor was pyocyanine produced when glucose, malate, or malonate were used as sole sources of carbon. Furthermore, malate even inhibited pigment production from substrates otherwise active.

A study on the role of metals was carried out in presence of the chelating agent Versene (ethylene diamine tetraacetic acid). Magnesium ions were shown to be essential and couldn't be replaced by manganese, iron, or cobalt. No need for the mentioned cations, except for magnesium, was demonstrated.

NaN_3 and KCN at a concentration of 0.01 M inhibited pigment formation altogether. ATP (0.01 M) was ineffective in restoring activity of KCN-poisoned cells. Iodoacetate (0.01 M) also prevented pyocyanine production, whereas NaF was not inhibitory even at a concentration of 0.01M.

The activity of glutamic acid, some dicarboxylic acids (in presence of ammonium), and inhibition by cyanide and azide, suggest that synthesis of pyocyanine is linked with the oxidative processes, presumably with the tricarboxylic acid cycle.

The Function of Blood in the Cultivation of Trypanosoma cruzi, N. CITRI and N. GROSSOWICZ, *Hebrew University — Hadassah Medical School, Jerusalem.* *Trypanosoma cruzi* is known to require blood for multiplication *in vitro*[1,2]. This requirement proved to be partly due to the need for exogenous haematin[3,4], but it was otherwise never explained. In order to make the analysis of other blood factors feasible, a vegetable product (tomato juice) was used to supply the essential nutrilites not specifically related to blood. This product, which served a similar purpose in nutritional studies on fastidious bacteria[5,6], was later successfully replaced by defined growth factors[7]. Serum albumin proved to be the only component of blood which could not be replaced[8]. The growth promoting effect of serum albumin was traced to its specific ability to bind and detoxify an essential lipid—oleic acid. Lipid-free preparations of crystalline serum-albumin were devoid of growth promoting effect, which however could be restored on addition of minute amounts of oleic acid. Thus the dual effect of oleic acid and the specific role of serum-albumin in detoxifying the essential lipid (previously recognized as a crucial factor in the growth of some bacteria [9-11]), seems to account fully for the blood or serum requirement of the trypanosome. Moreover, an explanation may be offered for the "dualistic" effect on *T. cruzi* by assuming that the cell permeability is influenced by the concentration of the free lipid; the minute amount of oleic acid supplied by the albumin complex is just sufficient to increase permeability so as to permit absorption of all essential nutrients. A further increase in permeability caused by excess oleic acid would adversely affect the cell and eventually cause lysis.

The "permeability regulating" function of the albumin-oleate complex might be of significance in the nutrition of other blood or serum dependent cells. Such possibility is stressed on account of the striking similarity in behaviour of the red blood cell and the trypanosome in relation to the albumin-oleate system.

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Serology

Some Aspects of the Middlebrook-Dubos Haemagglutination Test, M. SCHNITZER, U. BACHRACH and J. GUREVITCH, *Department of Clinical Microbiology, Hebrew University — Hadassah Medical School, Jerusalem.* Sheep erythrocytes were sensitized with two antigens: (a) Old Tuberculin, Lederle, and (b) a polysaccharide fraction isolated from Old Tuberculin, Welcome. Sera were examined from 32 tuberculin positive cows and from 16 cases of leprosy; they were inactivated and twofold serial dilutions tested against both types of sensitized cells. In each case the cells sensitized with Old Tuberculin, Lederle, gave a higher titre (1—2 tubes higher with the bovine sera and 3—4 tubes higher with the leprosy sera). The polysaccharide fraction, when dried, retained its activity for at least several months, while the sensitized cells lost theirs within 3 days.

Seven positive and four normal bovine sera were tested with differing concentrations of sensitized cells, and the optimal concentration of the latter was found to be between 0.2—0.3%. Lower concentrations gave agglutination with normal sera, and higher concentrations showed positive results only in lower titre.

The sensitivity of the haemagglutinins to temperature was studied and they were found to be destroyed at 63 °C in 30 minutes. At —4 °C the activity was preserved for several months.

Rabbits injected with live *Mycobacterium tuberculosis H37Rv* intravenously showed haemagglutinins already after one week. The titre rose to a maximum after 2—3 weeks and fell thereafter. In guinea pigs infected intraperitoneally with the same organism, on the other hand, antibodies did not appear until the second week, and then only in low titre. Autopsy showed that haemagglutinins appeared before the specific pathological changes were seen.

ABO Groups in Blood Platelets, J. GUREVITCH and D. NELKEN, *Hebrew University - Hadassah Medical School, Jerusalem.* We were able to demonstrate that suspensions of blood platelets in normal saline are agglutinated by *A*, *B*, and *O*-sera in the same way as these sera agglutinate red blood cells of the corresponding blood group. It was thus possible to establish four blood groups for the blood platelets, corresponding to the 4 major blood groups of human erythrocytes.

The following technique was used in the study: all glassware was coated with silicone; disodium-sequestrene was used as the anticoagulant, and Tritone (W.R. 1339 1%) was added to prevent irreversible clumping of the platelets during centrifugation.

Blood freshly drawn into siliconized tubes was centrifuged for 5—7 minutes at 800 rpm. The plasma was pipetted off and centrifuged again for 5 minutes at 800 rpm in order to remove the remaining red and white blood cells. Plasma containing a pure suspension of thrombocytes was now centrifuged 5 minutes at 2000 rpm, the supernatant plasma was siphoned off, a white pasty mass of platelets remaining on the bottom of the tube. Normal saline was added, and, by gently shaking, a homogeneous suspension of platelets was obtained. The suspension was made to contain an average concentration of 1—2 million platelets per mm^3.

The typing of the blood platelet suspensions was performed as follows: to 0.3—0.5 cc of inactivated high titered antiserum an equal volume of the platelet suspension was added and the whole was left in the refrigerator for 3 hours. Results were then read microscopically by withdrawing several drops of the serum-platelet suspension and spreading on a slide. Agglutinates of different sizes, from several to tens of thrombocytes, and large clumps of platelets, were clearly visible at low magnification.

Macroscopic readings followed by microscopic readings were repeated every 3 hours up to 24 hours, after gently tapping the tubes.

Platelets from 40 different blood samples were examined and all corresponded to the erythrocyte grouping.

The following table illustrates agglutination of blood platelets by *A*, *B*, *O*, and *AB* sera.

Platelets from blood of group	*A serum*	*B serum*	*O serum*	*AB serum*	*0.85% NaCl*
O	—	—	—	—	—
A	—	+	+	—	—
B	+	—	+	—	—
AB	+	+	+	—	—

\+ Agglutination
— No agglutination

When isoagglutinins in antisera were adsorbed by red blood cells, the adsorbed sera did not agglutinate thrombocytes. Similarly, when the same antisera were adsorbed by large amounts of platelet concentrates, the isoagglutinins were exhausted, so that these sera did not agglutinate red blood cells.

It was found that agglutination of platelets by antisera proceeds more rapidly at 4—6 °C than at 20 or 37 ° C.

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The Action of Polylysine on the Clotting of Plasma Induced by Staphylocoagulase, NOEMI BIEZUNSKI, E. SHAFRIR and A. DE VRIES, *Department of Clinical Research, Hebrew University—Hadassah Medical School, Jerusalem,* E. KATCHALSKI, *The Weizmann Institute of Science, Rehovot.* The coagulation of blood by staphylocoagulase proceeds in two stages: a) the enzyme staphylocoagulase reacts with the coagulase reacting factor

(CRF) present in plasma to form coagulase-thrombin, b) the coagulase thrombin clots fibrinogen tofibrin.

Staphylocoagulase was obtained in partially purified form from *Micrococcus pyogenes* var. *aureus* by the method of Duthie and Lorenz, utilizing cadmium sulfate as an enzyme adsorbant. It was found that the basic polyaminoacid, polylysine, accelerates clotting of plasma by staphylocoagulase, while lysine monomers, neutral polyalanine, and the acidic polyaspartic acid were without effect.

In order to find the site of action of polylysine, a clotting system composed of purified fibrinogen, partially purified staphylocoagulase, and CRF, was used. The accelerating action of polylysine has been shown to affect the second stage of the staphylocoagulase clotting system, i.e. the clotting of fibrinogen by coagulase thrombin. In the attempt to detect a possible action of polylysine on the first stage of the staphylocoagulase clotting reaction, use was made of the ability of polyaspartic acid to neutralize the activity of polylysine. When coagulase thrombin was produced in the presence of polylysine, no clot accelerating effect was observed when the polylysine was neutralized with polyaspartic acid before the addition of fibrinogen. This seems to indicate that polylysine does not affect the first stage of the staphylocoagulase clotting reaction.

Polylysine is known as a retarder of various biological reactions. It has antithromboplastic and antifibrinolytic activity, it inhibits the clearing of alimentary lipemia by heparin, it has virostatic and bacteriostatic activity, and it agglutinates red blood cells.

It seems that this is the first case in which polylysine acts as an accelerator of an enzymatic system.

The Complement Fixation Test in Bilharziasis, M. Eliakim and A. Michael Davies, *Hebrew Univerity — Hadassah Medical School, Jerusalem.* The complement fixation test is recognized as a valuable adjunct in the diagnosis of bilharziasis. Antigens have been derived from four main sources:

(a) the hepatopancreas of the infected intermediate host (snail),

(b) cercariae,

(c) adult schistosome worms, mainly *S. mansoni*,

(d) other trematodes (e.g. *Fasciola hepatica*).

Extracts of worms of *S. mansoni* and *F. hepatica* were made in buffered phenol saline (Coca's solution) and, together with an alcoholic extract of the livers of infected *Astrolorbis glabratus* snails, were tested out on the sera of patients with chronic bilharziasis and on those of healthy controls. The schistosome antigen gave a correct diagnosis in 86.5% of the cases and only 0.3% "false positive" results; the snail liver extract gave positive results in 54% of patients' sera, and the titre of the fasciola worm extract was too low for routine use.

In an attempt to increase the specificity and titre of antigens from schistosome worms, a number of different extracts were made and compared. Antigen was found only in extracts made in Coca's solution and absolute alcohol, and not in ether, acetone or formamide (polysaccharide) extracts. An extract in Coca's solution from the alcohol—ether insoluble fraction of the worms showed low antigenic property, but an alcoholic extract of the acetone insoluble fraction ws highly potent. Equally potent was that fraction of the alcohol—ether extract which could be precipitated by acetone. Of all these extracts, that in Coca's solution was the most specific, diagnosing correctly 83% of untreated patients. Other figures were 42% for the alcoholic fraction of the acetone insoluble residue, 13% for alcohol—ether extracts and 4% for residue. The suggestion is made that the active antigen is lipo-protein in nature.

Various techniques of extraction in Coca's solution showed that the most potent material was obtained by extraction of a powdered worm suspension in 500 parts of solution for an hour at 37° followed by 23 hours extraction at 28 °C. Second and-third extractions also yielded antigen but in falling titre. Extractions for various times at 2°, 56° and 100 °C, yielded less potent antigens.

Prepared Coca extracts retained their titre for a month when kept in the refrigerator with and without the addition of 0.1% cysteine. Freezing and lyophilization of the extracts caused a rapid drop in titre. Serial tests on fresh extracts of lyophilized or refrigerated thoroughly dessicated worms showed that the titre was retained for 4—6 months.

Thus, an antigen freshly prepared in Coca's solution from dry *S. mansoni* worms preserved in the cold is recommended as most suitable for use in the complement fixation test in bilharziasis.

Chemotherapy and Disinfection

Some Biological Properties of the Antibiotic Bacillomycin R, Ruhama Turner-Graff, J. Babad, A. Pinsky and N. Sharon, *Dairy Research Laboratory, Agricultural Research Station, Rehovoth.* The biological properties of bacillomycin R[1,2] were investigated. Different substances and inoculation conditions were tested in order to achieve maximal production of the factor. The antibiotic potency was tested by the Turner-Graff micro-technique[3], using Petri dishes and small test tubes with the substrate in double concentration. The test organism was *Penicillium roqueforti*. In the determination of the antimicrobial spectrum of the bacillomycin R, the following

groups were investigated: ascomycetes, including yeasts and molds with asci in perithecia, *Fungi imperfecti*, including members of the family *Hyphomycetales*, *Rhizoctonia*, and dermatophytes.

Experiments to elucidate the mechanism of action of the bacillomycin R were conducted The antibiotic was found to be fungistatic and, occasionally, after prolonged incubation with the test organism, fungicidal. The respiration of mold spores was not inhibited. On the contrary, a slightly increased oxygen uptake was observed. Microscopic examination showed that the bacillomycin R inhibits cell division and causes pronounced swelling of the spores. Growth of the molds under anaerobic conditions was not inhibited in the concentrations tested. The effects of the bacillomycin R on animals and human subjects are under investigation. A complete report is in preparation.

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The Antibacterial Action of Spermidine, S. Razin and R. Rozansky, *Hebrew University—Hadassah Medical School, Jerusalem.* Spermidine, like spermine, exhibited a bactericidal action against various microorganisms.

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Conditions which Determine the Efficiency of Ammonium Sulphate in the Control of Prymnesium Parvum in Fish Breeding Ponds, M. Shilo and Miriam Shilo, *Hebrew University—Hadassah Medical School, Jerusalem.*

Biosynthesis of Pigments of Ps. indigoflora, B. Volcani, *The Weizmann Institute of Science, Rehovot.*

Influence of Polypeptides on the Properties of the Bacterial Surface, L. Bichovski-Slomnitzki and E. Katchalski *The Weizmann Institute of Science, Rehovot.*

The Potentiating Effect of Purines on Sulfonamide Inhibition of E. coli, N. Grossowicz and Rachel Berger, *Department of Bacteriology, Hebrew University - Hadassah Medical School, Jerusalem.* Adenine was found to increase the inhibitory effect of sulfathiazole in *Escherichia coli*, thereby, confirming findings of Kohn and Harris[1].

To the compounds potentiating the effect of sulfathiazole belong besides adenine, hypoxanthine, guanine, also adenosine, adenylic acid and both ribose- and desoxyribose nucleic acids. Guanosine, guanylic acid and inosine, were less active. The effect was not shown by xanthine and the various pyrimidines. The active purines and purine derivatives were not inhibitory alone. None of the compounds showed any antisulfonamide activity as in case of lactobacilli[2,3].

The inhibition caused by sulfathiazole purine combinations was counteracted by *p*-aminobenzoic acid and by methionine, whereas vitamin B_{12} and folic acid were inactive.

Sulfonamide inhibition was often explained by interference with nucleic acid metabolism of *E. coli* cells[4,5]; the theory is based on the accumulation, in sulfa-treated cultures, of a purine precursor—4-amino-imidazole carboxamide.

In view of the augmenting effect exhibited by various purine derivatives and nucleic acids instead of counteracting it (as expected from the above theory), it is suggested that sulfathiazole inhibition is not due to a block of nucleic acid synthesis in *E. coli;* the accumulating carboxamide arises, presumably, by degradation of the purine under effect of the sulfa drug.

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Therapeutic Action of Chloromycetin and Aureomycin on Suckling Mice Following Oral Infection with Vibrio cholera, S. Davidovitch, *Israeli Institute for Biological Research, Ness Ziona* and *The Hebrew University — Hadassah Medical School, Jerusalem.* As a result of our search for a suitable experimental animal, a technique for feeding suckling mice with various strains of *V. cholera* has been evolved. Since infection of animals by the intraperitoneal route causes a disease altogether different from cholera in man, it was hoped that the development of a method of oral infection might form a basis for a therapeutic research.

Oral infection of young mice was tried, using 16—20 hr. old cultures on blood agar. The orga-

nisms were suspended in physiological saline containing 2% Na bicarbonate and 0.5% saponin.

5—6 days old suckling mice were sensitive to feeding of certain strains of *Vibrio*, and it was possible to determine LD_{50} by this route.

Tests were carried out to investigate the action of chloromycetin and aureomycin against oral infection of suckling mice with Inaba Strain 35 and 425-52 Ogawa strains. The oral dose per mouse was 5×10^7—5×10^8 vibrios. A single dose of the antibiotic was given in physiological saline by intraperitoneal or oral route, 3—4 hours or 24 hours after the oral infection.

Chloromycetin and aureomycin were both effective against oral infection. The minimal dose of chloromycetin required for curing infection was:

Effect of chloromycetin and aureomycin on oral infection of suckling mice with V. cholera

Substance tested	*Time after oral infection (hours)*	*Administration route*	*Minimum amount required for curing more than 50 % suckling mice (mg/kg)*	
			Inaba 35 strain	*Ogawa 425—52 strain*
Chloromycetin	3—4	intraperitoneally	10	60
	24		20—40	80
	3—4	orally	80	200
	24		200	200 not effective
Aureomycin	3—4	intraperitoneally	4	40
	24		60	60
	3—4	orally	20	60
	24		100	100 not effective

Techniques and Methods

Methods for the Regeneration of Solid Culture for the Growth of Serratia marcescens, I. HARTMAN, *Israeli Institute for Biological Research, Ness Ziona* 1954, *Harefuah*, **64**, 141.

Calculation of the Theoretical Error in Bacterial Count by Dilution and Plate Pouring, M. LEON, *Israeli Institute for Biological Research, Ness Ziona.*

Vitamin B_{12} Assay with a Mutant Strain of E. coli, J. ARONOVITCH, M. RACHMILEWITZ and N. GROSSOWICZ, *Departments of Bacteriology and Medicine "B", Hebrew University — Hadassah Medical School, Jerusalem.*

Bacteriological Examination of Hyaluronidase in the Tissues of the Eye, G. MEIR and J. Z. MICHAELSON, According to literature, hyaluronic acid has been found in the eye in considerable quantities in the vitreous and in the cornea, and the enzyme — hyaluronidase — was found in the iris and ciliary body. Based on these findings many theories have been propounded in connection with the physiological importance of hyaluronidase in the eye, and particularly on its role in certain pathological conditions, e.g. new vessel formation.

To confirm these theories a most sensitive test is required in order to prove the presence of very small quantities of hyaluronidase in the tissues of the eye.

The methods for estimation of hyaluronidase are:

1) turbidimetric method, 2) mucin clot prevention, 3) viscosimetric method, 4) microscopic method as reported by Murray and Pearce, and McClean.

The first three methods are not sensitive enough for our purpose.

The fourth method is based on the observation that certain strains of streptococci of the groups A and C, under certain conditions produce a capsule consisting of hyaluronic acid. This capsule is dissolved in the presence of hyaluronidase.

On repeatedly using the method of Murray and Pearce, using the same concentration of hyaluronidase each time, a greatly variable degree of enzymatic activity was found.

In our laboratory there has been developed a microscopic and macroscopic method which is a variation of the fourth method described above. This method makes possible the estimation of hyaluronidase in very small concentrations. A description of these methods is given in detail in the present paper.

Examining extractions of rabbits' irises, we were so far unable to confirm the presence of the enzyme in this tissue. On the rare occasions where hyaluronidase activity seemed to have been established, this was found to be due to contamination with bacteria producing hyaluronidase, and not to the presence of hyaluronidase in the rabbits' irises.

Phagocytosis of B.C.G. Bacilli by Cells in the Peritoneal Fluid of Guinea Pigs, Y. STEIN and R. BLUMENSTREICH, *Epidemiological Laboratories, Ministry of Health, Tel Aviv.*

Problems of Standardization of B.C.G. Vaccines, Y. STEIN, *Epidemiological Laboratories, Ministry of Health, Tel Aviv.*

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